University of Alberta

Investigation of Mould Contamination in Edmonton Public, Elk Island Catholic, and Elk Island Public Schools

by

Corinne Chantal Stocco

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

An indoor air quality study was conducted in 43 schools within the Capital Health Authority region in order to obtain baseline levels of airborne fungal contaminants. Samples were collected onto Rose Bengal agar in a total of 202 classrooms over fall 2001 and winter 2002 using a Reuter Centrifugal Sampler. The samples were analyzed by a mycologist at the Provincial Laboratory of Public Health for Northern Alberta (Edmonton). Fall indoor fungal counts ranged from non-detectable to 650 colony forming units per cubic meter (CFU/m^3), whereas in winter, indoor levels ranged from non-detectable to 160 CFU/m^3. During both sampling rounds, classrooms in 14% of schools exceeded 1995 Health Canada guidelines for *Penicillium* of 50 CFU/m^3. Upon subsequent investigation of these schools however, *Penicillium* levels were found to be below the guideline, and therefore not considered to be a persistent problem. Otherwise, the majority of rooms sampled met Health Canada guidelines.

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LIST OF ABBREVIATIONS

ACGIH	American conference of governmental industrial hygienists
AIHA	American industrial hygiene association
ASHRAE	American society of heating, refrigerating and air-conditioning engineers
A_w	water activity
BDL	below detection limit
CHA	Capital Health Authority
CIWG-IAQ	California interagency working group on indoor air quality
CFU	colony forming units
DG-18	dichloran 18% glycerol
DRBC	dichloran rose bengal chloramphenicol
EICS	Elk Island catholic schools
EIPS	Elk Island public schools
EPA	Environmental Protection Agency
EPS	Edmonton public schools
FPCEOH	Federal-Provincial Committee on Environmental and Occupational Health
GSC	gravity settling culture
HEPA	high efficiency particle arrestance
HPLC	high performance liquid chromatography
HVAC	heating, ventilation and air-conditioning
IAQ	indoor air quality
IEQ	indoor environmental quality
IQR	interquartile range
GC-MS	gas chromatography mass-spectrometry
GSC	gravity settling culture
HREB	health review ethics board

MEA	malt extract agar
NIOSH	National Institute of Occupational Safety and Health
NY DOH	New York department of health
OAI	outdoor air intake
QA/QC	quality assurance and quality control
RB	rose bengal
RCS	reuter centrifugal sampler
SDA	sabouraud dextrose agar
TLV	threshold limit value
VOC	volatile organic compound
V_p	vapour pressure
WHO	World Health Organization

1.0 INTRODUCTION

1.1 Background

Indoor environmental quality (IEQ) refers to physical, chemical and biological characteristics of an indoor site. Physical conditions include parameters such as temperature, humidity, lighting, and noise. Chemical aspects can include radon, formaldehyde, carbon monoxide, carbon dioxide and volatile organic compounds (VOCs). Biological pollutants include particles such as viruses, protozoa, fungi, bacteria, animal dander, dust mites and pollen. Interest in microbial evaluation in indoor air began in the late 1950's when hospital patients who had undergone surgery began suffering from secondary infections (Nathanson, 1990). Since then, indoor air pollution has become an important public health issue for the following reasons. With the goal of reducing costs during the energy crisis of the 1970's, energy conservation programs were instituted in Canadian buildings. One method to save energy was to seal the buildings, thus reducing the air exchange between indoors and outdoors, which ultimately led to an accumulation of pollutants inside the buildings (Health and Welfare Canada, 1987). Also, studies in North America and Europe indicate that people spend more than 90% of their time indoors (WHO, 1988). Further, in situations that allow microbial particles to build up to infectious dose, the ventilation system can distribute the contaminated air to other parts of the building (Yeager, 1999). The World Health Organization (1988) states that a large portion of disease and absenteeism from work and school is associated with infections and allergic episodes caused by exposure to indoor air. Because these sicknesses are often due to biological contaminants in buildings, they can be reduced significantly through adequate attention to the quality of indoor air. Furthermore, technical advances have enabled scientists to detect even lower levels of chemical and biological materials (Brooks and Davis, 1992).

Acceptable indoor air quality (IAQ), which includes measurable IEQ parameters, is defined by ASHRAE (1989) to be "air in which there are no known contaminants at harmful concentrations, as determined by cognizant authorities, and

with which a majority (80%) of people do not express dissatisfaction". Recently, Statistics Canada reported a four-fold increase in childhood asthma cases in the past 20 years, which was partially attributed to a deterioration of IAQ (Fok, 2000). The United States Environmental Protection Agency has ranked indoor air pollution as a high human risk (EPA, 1998), and the World Health Organization has recognized it as a serious threat to human health (WHO, 1998).

It is important to be aware that not all people share the same views listed in the sources above. Gots (1999) for example, believes that the exponential increase in interest into IAQ over the past few years is related less to an increase in understanding than to the misperception, mischaracterization, and exaggeration of a problem. He further maintains that the widespread state of high anxiety over indoor air developed due to the death of 29 people who attended a 1976 convention at a hotel in Philadelphia (Gots, 1999). Their deaths were eventually linked to the hotel's air-conditioning system which was circulating the bacterium *Legionella pneumophila* (Gots, 1999).

Edmonton Public Schools (EPS), Elk Island Catholic Schools (EICS), and Elk Island Public Schools (EIPS) are within the Capital Health Authority (CHA) region. Along with CHA, these organizations partnered with the University of Alberta to measure baseline levels of several IAQ parameters. EPS, EIPS, EICS are home to over 100,000 children in kindergarten through grade twelve, and to 6,500 teachers, administrators, and support staff. Although it is not believed that there are widespread problems within Capital region schools, this study was established for several reasons. First, school IAQ affects student health because children spend about 15% of their time at school and because they are more vulnerable than adults are to environmental contaminants. This is due to their greater breathing and metabolic rates relative to their size (CIWG-IAQ, 1998). Children are also more sensitive because their tissues and body systems are still developing. Recent studies have reported that IAQ also affects children's health and performance at school. For

example, poor IAQ can cause illness requiring absence from school and can cause acute health symptoms that decrease performance while at school (EPA, 2000b). Second, between 1986-1995, the IAQ of 110 federal government buildings in Canada was investigated, thus creating a large data set which in turn allowed the development of Health Canada guidelines (Minister of National Health and Welfare, 1995). In 1995, Health Canada's Environment Health Directorate further recommended that baseline levels of fungal contamination within public buildings be established. And third, a small number of schools within the Capital region have had IAQ problems, including inadequate ventilation and the presence of mould (Unland, 2001).

A review of 450 publications completed by Daisey and Angell (1999) indicated that the most commonly measured air pollutants in schools were VOCs, formaldehyde and microbiological pollutants. Although literature dealing with IAQ in schools has been abundant across North America and Europe, it is impossible to generalize the results of these studies due to different climates, different building structures, and varied experimental procedures.

The costs of poor school IAQ, which include poor health, reduced learning, and increased frustration when IAQ problems become unmanageable (CIWG-IAQ, 1998), are considerable and are paid for by students, staff, parents, and the community. CHA believes that this project will improve the local community's quality of life through the following benefits (Fok, 2000):

- *Reduced exposure to poor IAQ leads to healthier students and staff;*
- *Decreased financial burden on the healthcare and education systems through a reduction in the number of sick days;*
- *Increased school staff and public awareness of the effects of poor IAQ;*
- *Emphasis on proactive management of school IAQ;*
- *Development of a cost effective fungal management strategy.*

[Italics added]

1.2 Research Objectives

This report, which deals with fungal contaminants, is one of a three part series which establishes a baseline set of data in CHA schools, for several IAQ parameters. A second report deals with ventilation and comfort parameters including temperature, humidity, carbon dioxide and carbon monoxide, while a third report addresses correlations between ventilation and comfort parameters and fungal contamination.

The purpose of this report is to describe the relevance of mould in indoor air, to explain the experimental design and the mould sampling procedures that were undertaken during collection of bio-measurements, as well as to describe fungal levels that were obtained in 43 schools across the Capital Health region. This baseline set of data, together with a standardized method of fungal sampling will also help schools to determine whether fungal problems exist in schools with indoor air quality complaints in the future.

2.0 LITERATURE REVIEW

2.1 Fungi

Along with bacteria, viruses, amoebae, pollen and house dust mites, fungi are classified as microbiological pollutants. Fungi can further be broken down into moulds, yeasts, mildews, large mushrooms, puffballs, bracket fungi, rusts and smuts. Presently, there are 1,000,000 known species of fungi and scientists estimate that an additional 2,000 new species are identified each year (Natural Academy of Sciences, 2000; Starr and Taggart, 1995).

Fungi are mostly multicellular, eukaryotic heterotrophic organisms. Most fungi are saprobes, obtaining nutrients from non-living organisms, however some are parasites and extract nutrients from living hosts. Some fungi are even symbiotes, living in a mutualistic environment with another living organism. Saprobic fungi are responsible for most natural aerobic decay and nutrient recycling (Burge and Otten, 1999). Because they cannot manufacture their own food, fungi secrete digestive enzymes onto organic matter and then absorb the broken down sugars and biological materials (Starr and Taggart, 1995).

It is not only as sources of food (mushrooms, yeast) for human consumption that fungi are of economic importance. Fungi are producers of antibiotics such as penicillin, but are also major deteriogens to farmers' crops and manufacturers of food such as bread and fruits. Gravesen et al., (1994) state that there are 50 fungal genera that are relevant to food spoilage including *Aspergillus*, *Penicillium*, and *Fusarium*. It is hypothesized that there is at least one fungus that can digest any organic-containing substance (Miller, 1992). Fungus can grow on and digest jet fuel, paint, rubber, textiles, electrical equipment, paper, cardboard, ceiling tiles, cloth, fabrics, twine, leather, and wood products (Miller, 1992). If the air is sufficiently humid to permit them to grow rapidly, moulds can even corrode the lenses of binoculars, telescopes, and microscopes (Christensen, 1951). Metals, which were previously thought to be exempt (Christensen, 1951) can also be attacked by fungi (Ahearn et al., 1991).

In the process of degrading substrates, fungi produce many metabolic products, the principal ones being carbon dioxide and water. Although most fungi carry on aerobic respiration, some like yeast, can ferment yielding lactic acid or alcohol (Atlas, 1995; Burge, 1990). The reactions are as follows:

Classic Aerobic Respiration:

$$C_6H_{12}O_6 + 6O_2 + 2ADP + 2P_i \rightarrow 6CO_2 + 6H_2O + 2pyruvate + 2ATP \quad [1]$$

Homolactic Fermentation:

$$C_6H_{12}O_6 + 2ADP + 2P_i \rightarrow 2Lactic\ Acid + 2ATP \quad [2]$$

Heterolactic Fermentation:

$$C_6H_{12}O_6 + ADP + P_i \rightarrow Lactic\ Acid + CH_3CH_2OH + CO_2 + ATP \quad [3]$$

Ethanolic Fermentation:

$$C_6H_{12}O_6 + 2ADP + 2P_i \rightarrow 2CH_3CH_2OH + 2CO_2 + 2ATP \quad [4]$$

Most fungi form long chained, tube-shaped hyphae (a mass of them is called a mycelium), that are chitin-reinforced and that allow nutrients to flow freely throughout (Starr and Taggart, 1995). Studies indicate that, given plenty of food and no competition, a moderately fast-growing fungus will cover the surface of a 3 inch-wide culture dish in three or four days, advancing at the rate of about 310 μm/min (Christensen, 1951). This rapid growth of mycelium explains why moulds can invade materials rather quickly when conditions for their growth are favourable.

Mycologists classify fungi based on the organisms lifecycle; they can reproduce both sexually and asexually. The four major groups are: zygomycetes, ascomycetes (sac fungi), basidiomycetes (club fungi), and the deuteromycetes (imperfect or asexual fungi) (Burge and Otten, 1999; Atlas, 1995). During both asexual and sexual phases of reproduction, various spores may be produced, however

Burge (1990) states that the former phase contributes more significantly to indoor air pollution because it contains genera such as *Penicillium*, *Aspergillus*, and *Alternaria*.

A spore, which has been highly adapted for survival and dispersal, is a discrete reproductive unit, usually enclosed by a rigid cell wall that protects against desiccation and is often pigmented to prevent ultraviolet damage (Gravesen et al. 1994; Moore-Landecker, 1982). The hardiness of the spores are also described by Miller (1992) who maintains that spores of *Aspergillus* and *Penicillium* species can remain viable for more than 12 years at dry room temperature conditions. Spores range in size from 2-100 μm (Burge, 1985) and are released into the air by raindrops, animal movement and air currents. Spores have been known to travel dozens of kilometres before reaching their final destination (Miller, 1992) and because spores are also very light, they settle as a function of their mass to volume ratio. In closed tube experiments, Christensen (1951) found that spores are so buoyant that a beam of light projected through the tube in which spores were falling created enough air current to send them upwards. Larger disturbances of fungal spores such as vacuuming a carpet will release millions of spores, which may have an effect on IAQ (Miller, 1992). Table 1. presents several fungal types according to their mycological classification, and also includes, as previously mentioned, their specific economic importance.

Table 1. Some Economically Important Fungi

Classification	Application or Consequence
Basidiomycetes	
Puccinia graminis	Black stem wheat rust
Uromyces species	Bean rust
Ustilgo maydis	Smut of corn
Amanita phalloides	Usually fatal if ingested
Tilletia species	Smut of wheat
Urocystis species	Smut of onion

Table 1. Some Economically Important Fungi (Con't)

Classification	Application or Consequence
Zygomycetes	
Rhizopus species	Food spoilage
Mucor species	Opportunistic human pathogen
Ascomycetes	
Saccharomyces cerverisiae,	Used as baker's yeast
Saccharomyces carlsbergensis	Used to ferment wine, beer and spirits
Claviceps purpurea	Ergot of rye
Endothia parasitica	Chestnut blight
Ceratocystis ulmi	Dutch elm disease
Emmonsiella capsulata	Histoplasmosis in humans
Morchella esculanta	Gastronomic delight
Venturia inaequalis	Apple scab
Monilinia fructicola	Brown rot of stone fruits
Deuteromycetes	
Penicillium species	Penicillin antibiotic
Aspergillus species	Used in blue cheese production
Trichoderma species	Toxigenic
Alternaria species	Common allergen
Cladosporium species	Common allergen
Epicoccum species	Common allergen
Fusarium species	Toxigenic

(Adapted from Atlas, 1995; Starr & Taggart, 1995; Etkin, 1994)

2.2 Mould in the Indoor Environment

2.2.1 Factors Affecting Growth

Fungal growth and reproduction are affected by a number of factors including nutrient availability, substrate composition, air velocity, relative humidity and pH, however moisture availability and temperature are heralded as the two major factors (Blackburn, 1997; Burge, 1985). Many authors state that it is actually the water activity (A_w) of a building material, rather than the relative humidity of a room, which is the important determining factor of mould growth in indoor environments (Cooley et al. 1998; Gravensen et al., 1994). According to equation [5], A_w is the expression

of the substrate's available moisture at equilibrium (Atlas, 1995; Miller, 1992). A_w values are given for certain fungal groups in Table 2.

$$A_w = V_p \text{ of water in substrate} / V_p \text{ of pure water} \quad [5]$$

where V_p is the vapour pressure

Table 2. Limiting Water Activities for Fungal Growth

Fungal Group	Water Activity (A_w)*
Fusarium	0.90
Mucor	0.90
Yeasts	0.88
Penicillium	0.80
Aspergillus	0.75

*Approximate value (Atlas, 1995; Moss, 1987)

Temperature, which also affects fungal growth, does so mostly through its control over the water activity of the substrate (Burge and Otten, 1999). Fungi have a wide temperature range for growth and belong to one of three categories: thermophiles, mesophiles, or psychrophiles (Table 3). In the context of this research, fungi found indoors are mainly mesophilic and prefer the slightly acidic conditions of pH 5-6 (Gravesen, 1979).

Table 3. Optimal Fungal Growth Temperatures

Category	Temperature (°C)
Psychrophiles	0 - 20
Mesophiles	15 - 30
Thermophiles	35 - 50

(Gravesen, 1979)

2.2.2 Sources of Indoor Mould

As indicated by the sheer number of species, fungi are ubiquitous in nature. Studies reveal that even control buildings, which were determined as not having mould problems, always have some baseline level of fungal contaminant (Hyvärinen et al. 1993) because of outdoor mycoflora permeating indoor air. In fact, most spores

that are present indoors originate from the outdoors (Pasanen et al., 1992). Fungal spores are found in offices, homes, schools, hospitals, shopping malls and churches, and are brought inside by track-in on shoes and clothes, by repeated water damage, and by the circulation through the ventilation system, open windows, and air leaks. Other minor natural sources of indoor fungi include rotting food, plants, and pet bedding materials (WHO, 1988).

A recent report by the Lawrence Berkeley National Laboratory reviewed the literature on California school IAQ and found that the most common building-related problem was inadequate ventilation with outside air (CIWG-IAQ, 1998). In brief, ventilation systems mix outdoor air with recirculated air and then distribute it to the occupied environment (ACGIH, 1989). Although ventilation systems can play a role in achieving good IAQ by diluting air contaminants, they can also be a contributing factor to the deterioration of IAQ by generating or distributing air contaminants (Bearg, 1993). Due to its relevance to IAQ, a brief overview of the typical components of a heating ventilation and air-conditioning (HVAC) system (Figure 1) is included, as well as a discussion of potential aerosols that can be associated with each component.

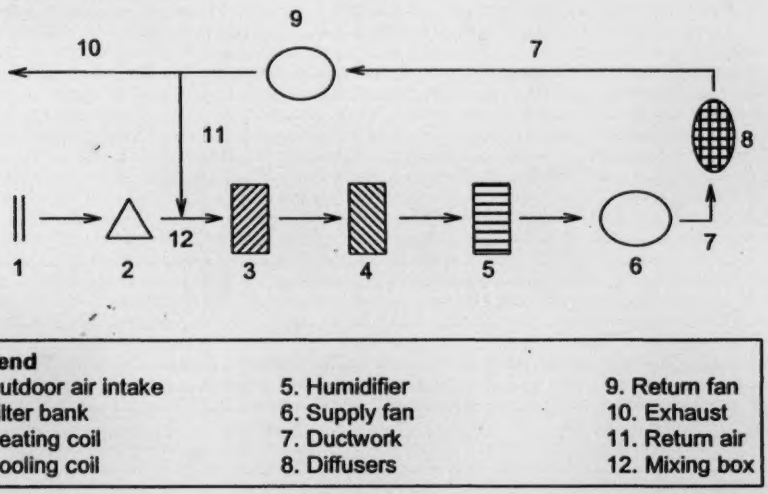


Figure 1. Schematic of an HVAC System (Adapted from ACGIH, 1989)

Fresh air is brought in through the outdoor air intake (OAI) and is transported to the mixed air plenum. Pools of water, leaves, or soil near the OAI can support the growth of microbials, which then have easy access into the building. More seriously, bird droppings near OAIs can harbour the infectious fungi *Emmonsiella capsulata* (previously classified as *Histoplasma capsulata*) and *Cryptococcus* (ACGIH, 1989), which is why bird screens, which deter roosting birds, are common to most OAIs. Once inside, outdoor air is mixed with a portion of recirculated indoor air, and then is carried through a series of filter banks. Properly functioning filters look somewhat dirty on their upstream side because, as a filter loads up, the pressure drop across it increases and thus the filtration efficiency also increases (Bearg, 1993; ACGIH 1989). Because the volume of air passing through the filters decreases over time, they are regularly changed. Tight-fitting filters are essential as gaps between filters will permit unfiltered air to penetrate the rest of the system (Bearg, 1993).

After filtration, the mixed air enters the heat exchanger, which consists of heating and cooling coils that help maintain comfortable thermal conditions within the building. If air is cooled beyond its dew point, moisture condenses on the exterior of the cooling coils and the water drains into a pan (Brooks and Davis, 1992). If the pitch of the drain pan is inadequate or if the drain becomes clogged, stagnant water can accumulate thus making an ideal reservoir for microbial contaminants. Next, the supply fan pushes the air into individual rooms via supply ductwork. Although ductwork is never sterile, it should not contain a thick layer of dirt where fungal growth can occur. The return fan then draws air from the rooms into ductwork where a portion of the air is exhausted to the outdoors, while some of it is recirculated through the system.

The system described above is more likely to be found in high schools, whereas in elementary schools air is usually supplied by unit ventilators which are located in each classroom (Johnston, 2001). Unit ventilators consist of an OAI, a mixing plenum, a filter, and a heater. These less complicated systems are readily

found in older elementary schools because their technology is older and because their capital and their maintenance costs are lower than running the more complicated HVAC systems. Also, because there are usually fewer occupants per classroom, the air exchange rate is lower in elementary schools than in high schools and can adequately be provided by a properly functioning unit ventilator.

2.2.3 Risk Factors Which Make Schools Prone to Fungal Contamination

There are many elements that interact in a building to yield the overall condition of the indoor environment, some of which have already been discussed (for example, the ventilation system). Research has identified other key features that lead to poor air quality. These include an assortment of factors that can be categorized into building envelope, school location and repeated water damage.

The building envelope serves to insulate and to protect occupants from the elements (Bearg, 1993). Ellringer et al. (2000) state that common construction materials such as gypsum wallboard, fibrous glass insulation and vinyl wallpaper all contain nutrients that fungi can utilize. Steel or masonry materials like concrete and tile however, are more resistant to fungal colonization due to the minimal cellulose content. A separate study conducted by Rand (1998) which assessed mould contamination problems in Atlantic Canadian schools also found that spore burdens in wood frames were significantly higher than in steel frame and combination frames. IAQ studies in Connecticut have illustrated that schools built with poorly designed and maintained flat roofs, and those built on concrete slabs are particularly susceptible to moisture intrusion (Werle, 2000). A study conducted by Pasanen et al. (1992) indicated that spore counts increased with the age of a building. Also, portable classrooms present a unique set of IAQ problems, largely because of their inadequate ventilation systems (Werle, 2000), and because their life expectancies are often exceeded (Minister of National Health and Welfare, 1995). And finally, a 1987 Danish study found that carpeted rooms contain significantly more surface dust than do those without carpet (Etkin, 1994). Further to this, Gravesen et al. (1994)

demonstrated that the floor dust from carpeted rooms contains significantly higher colony counts of moulds, particularly *Penicillium, Cladosporium* and *Aspergillus*. Researchers have concluded that carpets act as both reservoirs and amplifiers for fungi, particularly after water damage. In comparison to bare floors, carpets tend to retain more moisture and are less easily cleaned of dust (Etkin, 1994).

Pasanen et al. (1992) found that spore counts in indoor and outdoor air are higher in rural than in urban environments, and hypothesize that agricultural sources and handling of organic debris increase the spore burden in the rural environment. If the school has had a history of repeated water damage, combined with warm temperatures, indoor sources of mould can develop. Pasanen et al. (1992) indicated that fungal microcolonies could develop within days on indoor surfaces that are occasionally wet.

2.3 Exposure to Mould

There are three pathways of human exposure to fungal spores: through ingestion, dermal contact, and inhalation (Burge and Ammann, 1999). In the context of this research, inhalation is the most significant route. Whether or not people exposed to fungi develop symptoms depends on the nature of the fungal material, the duration of exposure, the level of toxin intake, and the susceptibility of exposed persons (NY DOH, 2000; Ammann, 1999; Schiefer, 1990). Susceptibility varies with genetic predisposition, age, and state of health (NY DOH, 2000). As previously indicated, young children tend to be more sensitive than adults to irritating air contaminants (Werle, 2000).

Poor school IAQ can cause both short and long-term effects in students and staff (CIWG-IAQ, 1998), which are reversible and chronic respectively. Due to difficulties presented with epidemiological studies and lack of chronic data, it is difficult to determine exactly what effect fungal products have on humans. Principal systemic effects of inhaling fungal spores are associated with the lungs and the

immune system (Health and Welfare Canada, 1987). Direct inhalation depends on the size of the material (dust or spore). Particles with aerodynamic diameters greater than 5 μm may not reach the brochi or alveolar spaces (Schiefer, 1990). Exposure to airborne fungi may lead to increased fatigue, concentration difficulties, headaches, eye, skin and throat irritation, increased frequency of common colds, wheeze and skin sensitization (Whillans, 1996). Many of these non-specific health problems can also be associated with a plethora of conditions including personality traits, job dissatisfaction and hay fever (Gots, 1999). Risk of flu symptoms, diarrhea, dermatitis, malaise, epistaxia, hypersensitivity pneumonitis, and fever also increases in those exposed to mould (NY DOH, 2000; Minister of National Health and Welfare, 1995). Burge (1990) also states that some fungi such as *E. capsulata* and *Cryptococcus* are pathogens and will invade human tissues causing infectious diseases such as histoplasmosis and cryptococcosis respectively. Apart from the immunocompromised, the human immune system is well-equipped to prevent such invasions (Burge, 1990).

Fungi produce an amazing array of metabolites that can be categorized into VOCs, allergens, and mycotoxins. *Penicillium* species for example, biosynthesize approximately 380 metabolites (Mantle, 1987). Metabolites are not usually formed while the organism is in an active growth phase, but rather occur when growth limitation due to food, temperature, or pH lead to diversion from primary to secondary processes (Mantle, 1987). Many species of fungi that are common indoor contaminants produce these secondary products, which are often toxic, raising questions about possible health effects due to airborne exposure (Levetin, 1995). AIHA (1996) states that the combined effects of mould allergens and mycotoxins on the immune system may be additive or synergistic. Furthermore, fungi need not be alive and culturable to be antigenic and toxins remain in the environment long after the fungus is dead (Burge, 1990).

Species specific data can also make the difference between modest and major concern. For example, *Aspergillus niger* is a common spoilage organism and is not hazardous to health, whereas *Aspergillus fumigatus* is potentially harmful to human health as it produces potent mycotoxins such as glotoxin and tremorgenic toxins (Scudamore, 1994). Despite the benefits of species differentiation, it is difficult and costly to identify a fungus beyond its genus (Health and Welfare Canada, 1987).

2.3.1 Volatile Organic Compounds

Fungi produce VOCs during both primary and secondary metabolism (Ammann, 1999). Over 500 VOCs have been identified from various types of fungi (Miller, 1992). The production of VOCs cause the typical "mouldy" odour associated with fungal growth (Burge, 1990). Ethanol is the most commonly formed VOC, and is a potent synergizer of many toxins (Miller, 1992). In a study where the headspace gas of a culture of *Penicillium fellutanum* was analyzed by a gas chromatography mass-spectrometry (GC-MS) instrument, VOCs such as pentane, heptane, octane, methyl butanol, hexanone and heptanone were identified (Miller, 1992).

2.3.2 Mycotoxins

Mycotoxins are nonvolatile, low molecular weight secondary metabolic products that have deleterious effects on humans (Burge and Ammann, 1999). Mycotoxins themselves do not constitute a chemical category because they have no molecular features in common (Burge and Ammann, 1999). Their chemical structures vary from polyketides, to terpenes, to indoles. The two main types of mycotoxins produced by fungi are the tricothecenes and the aflatoxins. Research indicates that ingestion is the primary pathway for mycotoxins to enter the human body, and that people have died from eating grain products contaminated with fungi containing ergot alkaloids (Burge and Ammann, 1999). Currently, there is no information on the effects of chronic, low doses of mycotoxins from inhalation experiments (Miller, 1992). Acute inhalation exposure of mice, rats and guinea pigs to T-2 toxin (a trichothecene produced by *Fusarium*) however, was found to be 2 to

>20 times more toxic than the intravenously administered toxin (Creasia et al., 1990). It is difficult to extrapolate human risk from laboratory animal data because it involves several assumptions. First, that the effects on human cells will parallel those in animal models. Second, that natural human exposures are equivalent to those from laboratory animals. Third, that exposure of experimental animals is at much higher dose to achieve a response in a small exposure population over a short period of time (Burge and Ammann, 1999). Table 4 depicts mycotoxins produced by some common indoor fungi.

Table 4. Mycotoxins Produced by Some Fungus Found Indoors

Fungi	Chemical Metabolite	Health Effect
Alternaria	Altenuene	Cytotoxic
	Alternariol	Cytotoxic
	Alternariol methyl ether	Cytotoxic; teratogenic
	Altertoxins I,II	Cytotoxic; mutagenic
Aspergillus	Aflatoxin	Liver and respiratory cancer
	Endotoxin	Allergenic
	Ergot alkaloids	Neurotoxic
	Fumigaclavine A,B,C	Gastrointestinal toxicity
	Fumigatoxin	Respiratory toxicity; neurotoxicity
	Fumigillin	Gastrointestinal and dermal toxicity
	Fumitremorgen A,B	Neurotoxic
	Gliotoxin	Immunosuppresive
	Helvolic acid	Hepatotoxic
Cladosporium	Emodin	Reduced cellular oxygen uptake
Penicillium	Citrinin	Renal damage; vasodilation
	Gliotoxin	Lung disease
	Patulin	Hemorrhage in lung and brain
	Secalonic acid D	Lung toxin; teratogenic in rodents
	Verruculogen	Neurotoxin; trembling in animals
Stachybotrys	Deoxynivalenol	Bleeding; dermal necrosis
	Satratoxin H	Chronic low does is lethal; teratogen
	Sealenone	Alters immune function
	Spirolactone	Anti-complement function
	Trichothecenes	Immune suppression; cytotoxic

(Adapted from Etkin, 1994)

2.3.2.1 Tricothecenes

There are about 150 known tricothecenes, that are produced by various species of fungi including *Fusarium, Trichothecium, Trichoderma,* and *Stachybotrys* (WHO, 1990). An exhaustive literature search conducted by Nielsen (2001) determined that *Stachybotrys* species produced 18 tricothecenes, all of which are highly cytotoxic. Based on animal models, tricothecene mycotoxins may affect cellular membranes, inhibit synthesis of protein and nucleic acids, and adversely affect the lymphatic system (Andersson et al., 1997). Trichothecenes are immunosuppressive agents that can make animals susceptible to opportunistic bacterial infections (Croft et al., 1986) and are also very irritating to the skin, affect bone marrow, and promote changes in the heart rate (Schiefer, 1990).

Two Toronto Catholic primary schools closed because *Stachybotrys chartarum,* which may emit satratoxin, verrucarin, roridin, and stachybocin mycotoxins, was reported to have compromised the health of the students and staff. Occupants in both schools had long complained of headaches, respiratory problems and fatigue (Post, 1999; Yeager, 1999). Recently, infants in home environments, later found to contain strains of *S. chartarum*, were reported to be suffering from pulmonary hemorrhaging (Burge and Ammann, 1999).

2.3.2.2 Aflatoxins

Although the term aflatoxin (derived from *Aspergillus flavus* toxin) is the name given to twelve structurally related compounds, it usually refers to four metabolites containing a bisfurano-coumarin structure (Figure 2): Aflatoxin B_1, B_2, G_1 and G_2 (Dvorácková, 1990). These four major aflatoxins are produced by both *Aspergillus flavus* and by *Aspergillus parasiticus*.

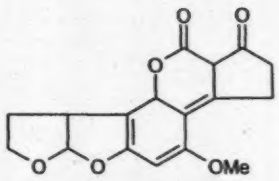


Figure 2. Chemical Structure of Aflatoxin B_2

The general public is now aware of the potential harm of ingesting aflatoxins B_1 and B_2, which are found in tainted oil seeds such as peanuts, pistachios, almonds, and walnuts. Occupational inhalation exposure to aflatoxins due to handling and processing of contaminated agricultural products has also been associated with increased risk of liver cancer (Cotty et al., 1994). Furthermore, aflatoxins are acutely toxic to the brain, kidneys and heart (Ammann, 2000), and can promote DNA damage (Scudamore, 1994).

2.3.3 Allergens

Between 6-15% of the Canadian population is allergic to fungi (Miller, 1992). As many as 4.5 million Canadians may elicit an inappropriate immune response to the inhalation of fungi because their bodies recognize the substance to be foreign. Most antigens are proteins with large molecular weights, and their ability to cause an immune response is related to the complexity of the molecule (Burge and Ammann, 1999). Fungal antigenic products, which include the organisms themselves, spores, and fragments are commonly found in outdoor air, settled dust, indoors and in humidifiers (Burge and Ammann, 1999). Once inhaled into the lungs, the body will respond to the antigenic material in a variety of ways which are not necessarily mutually exclusive: allergic asthma, rhinitis, conjunctivitis, skin rash, anaphylactic shock, cytotoxic reactions, immune complex reaction and lymphocyte-mediated immunity (Burge and Ammann, 1999). *Alternaria* is a common fungus that grows on living plants, straw, leaves, grass clippings, rotten fruit, and seeds, and is a common cause of respiratory allergy in human beings (Christensen, 1951).

2.4 Reported Background Numbers

The investigation of microorganisms in outdoor and indoor environments is not a novel undertaking. Many studies of this genre have been completed in Europe and North America, usually in response to complaints of IAQ problems. A review of the literature revealed that the majority of these investigations were completed in private residences, whereas fewer were performed in public buildings and in schools.

2.4.1 Outdoors

Burge et al. (1977) state that the concentrations of fungi in the outdoor air varies by several orders of magnitude and that air routinely contains greater than 1,000 spores/m^3. The number of spores and the type of species present outdoors are dependent on season, climate and weather conditions, as well as on human activities (WHO, 1997).

The results from a three-year study performed in Copenhagen revealed that most outdoor fungi show seasonal dependence (Larsen, 1981). From January to April the individual colony-counts of *Aspergillus, Cladosporium, Penicillium,* and *Alternaria* species stayed below 50 CFU/m^3. *Aspergillus* and *Penicillium* species were present in small quantities all year long, but the latter species became more frequent during the winter months. Although *Cladosporium* counts exceeded that of *Alternaria*, both species exhibited similar seasonal trends such as peaking in August. These results corroborate well with Canadian literature indicating that *Cladosporium* and *Alternaria* contribute 60-70% of spores in the air during the growing season (Minister of Health and Welfare, 1995).

The numbers of outdoor fungal spores are a function of the current weather, and Burge (1986) also purports that preceeding climactic conditions also has an affect on spore load. *Fusarium* and *Phoma* species, ascospores and basidiospores prevail in wet weather, while *Cladosporium, Alternaria,* and *Epicoccum* are most prevalent in dry, windy weather (Bush and Portnoy, 2001).

Soil and vegetation provide the most important sources of outdoor airborne fungi (WHO, 1997). When human activities such as harvesting stir up the soil, billions of spores are released per minute (Burge et al., 1977). Large numbers of spores are also released into the air when doing yard work such as raking leaves or cutting the lawn.

2.4.2 Indoors

Many studies have determined background levels of indoor contaminants in control buildings that have not had previous water damage or IAQ complaints. The first portion of information included below was mainly derived from non-problem public and private buildings, whereas Table 5 focuses on studies conducted in schools. Special focus should be placed on the school studies completed in Spokane, U.S. and in Alberta as their climates are similar to Edmonton.

In a 1991 study of 695 U.S. commercial buildings by the Business Council on indoor air, fungal growth was present in 35% of the buildings surveyed (Post, 1999). Hyvärinen et al. (1993) determined that the indoor air concentrations of viable fungi varied between 40-580 CFU/m^3 during the fall, and between 15-220 CFU/m^3 during the winter. With reference to the home, Health and Welfare Canada (1987) states that indoor air is likely to contain *Penicillium, Aspergillius, Cladosporium, and Alternaria* species. More recently, a study conducted by the FPCEOH (1995) in more than 50 government buildings, determined that the "normal" air mycoflora was approximately 40 CFU/m^3 for *Cladosporium, Alternaria*, and non-sporulating basidiomycetes. The microbial flora listed above are regarded as normal background components of indoor air, but some indicator organisms occur only in environments with moist materials. These include: *A. fumigatus, Aspergillus versicolor, Penicillium, Trichoderma, Eurotium, Exophalia, Phialophora, Stachybotrys, Fusarium, Ulocladium, Wallemia,* & yeasts (WHO, 1997).

Table 5. Summary of Fungal Contaminant Studies Conducted in Schools

Location	Number of Schools	Range (CFU/m^3)	Most Common Species
Paris, France [a]	10	BDL - 1000	(not given)
Spokane, US [b]	3	16 - 531	*Cladosporium*, basidiospores, *Penicillium, Aspergillus, Alternaria*
Kansas City, US [b]	4	136 - 4970	*Cladosporium*, basidiospores, *Penicillium, Aspergillus*
Santa Fe, US [b]	4	17 - 4130	*Cladosporium, Aureobasidium*, basidiospores, *Penicillium, Aspergillus*
Orlando, US [b]	2	76 - 6450	*Cladosporium*, basidiospores, *Penicillium, Aspergillus, Curvularia*
Atlantic Canada [c]	631	BDL - 350	*Cladosporium, Penicillium, Alternaria*
Vancouver, Canada [d]	39	(not given)	*Penicillium, Cladosporium*, yeasts, *Aureobasidium*
Edmonton area, Canada [e]	1	BDL - 176	*Penicillium, Aspergillus*

[a] Mouilleseaux et al., 1993

[b] Leventin et al., 1995

[c] Rand, 1998

[d] Bartlett et al., 1998

[e] Probert et al., 2000

Due to regular occupant activity, hourly variations of four times have been found in classrooms (Miller, 1992). Weekly variations of airborne propagules of between one and two orders of magnitude have been demonstrated in houses (Miller, 1992).

2.5 Reported Exposure Limits and Guidelines for Mould

There are no legally enforceable standards for fungal contaminants in indoor air (Werle, 2000). This is in part because no threshold limit value (TLV) can be established. Macher et al., (1995) provide several reasons why TLVs for fungal pollutants are not scientifically supportable: a) spores do not comprise a single entity, b) human responses to the spores vary, and c) measured concentrations of bioaerosols are dependent on the method of sample collection and analysis.

Currently, there are several guidelines that have been established for mould contaminants. A common approach for developing guidelines has been to accept a set of baseline data from a cross-section of samples as representing the "normal" indoor air spore load and to relate new data to the distribution of the baseline set (Rao et al., 1996; Yang et al., 1993). Table 6 summarizes guidelines put forth by major government and private organizations from around the world. When the actual source could not be obtained, a reference to Rao et al., (1996) is included as the source of information.

Table 6. Fungal Guidelines Put Forth by Various Organizations

ACGIH (1989)
Consensus-based guidelines.
- < 100 CFU/m^3 is acceptable
- indoor:outdoor <1 is acceptable if species are similar

ACGIH (2001)
Derived by a review of the literature.
- 100-1000 CFU/m^3 is considered intermediate contamination

AIHA (1996)
Based on ACGIH (1989) guidelines.
- Rank order assessment determines if indoor and outdoor taxa are similar

Commission of the European Communities (Rao et al., 1996)
Used data from residential IAQ studies in Europe and Canada which used N-6 Andersen samplers with either MEA or DG-18 agar.

	Non-industrial indoor:	Residences:
• very low:	< 25 CFU/m^3	< 50 CFU/m^3
• low:	< 100 CFU/m^3	< 200 CFU/m^3
• intermediate:	< 500 CFU/m^3	< 10^3 CFU/m^3
• high:	< 2000 CFU/m^3	< 10^4 CFU/m^3
• very high:	> 2000 CFU/m^3	< 10^4 CFU/m^3

Denmark (Heida et al., 1995)
Monitored fungal counts of a 3500 m^2 composting facility using an Andersen High Volume Sampler and MEA.
- > 10,000 CFU/m^3 in total is a threat to workers' health
- 500 CFU/m^3 of pathogenic species are a threat to workers' health

Table 6. Fungal Guidelines Put Forth by Various Organizations (Con't)

Canada Mortgage and Housing Corporation (Rao et al., 1996)
Used an RCS and RB agar to collect samples in 50 Canadian problem homes.

- ≥ 50 CFU/m^3 of one species may require further action
- ≤ 150-200 CFU/m^3 is acceptable if a variety of species are present
- ≥ 200 CFU/m^3 of several species may require further investigation
- 400-500 CFU/m^3 is ok if species are mainly *Cladosporium* and *Alternaria*
- ≥ 500 CFU/m^3 requires further investigation

National Health and Welfare Canada (1995)
110 buildings were investigated over nine years using and RCS and RB agar.

- Pathogenic and toxigenic fungi are not acceptable in indoor air
- ≥ 50 CFU/m^3 of one species may require further investigation
- ≤ 150 CFU/m^3 is acceptable if there is a mixture of species
- ≤ 500 CFU/m^3 is ok in summer if they are common tree or leaf fungus
- Indoor air is qualitatively similar and quantitatively lower than outdoor air
- Presence of fungi in humidifiers and on ducts, mouldy ceiling tiles and other surfaces requires remedial action

New York City (NY DOH, 2000)
Based on literature review and consultation with a panel of experts. Originally established for *S. chartarum* but has since expanded to include all fungi.

- 10^3-10^4 CFU/m^3 necessitates building evacuation
- The area of fungal growth determines the type of remediation:
  - Level 1: Small isolated areas ≤ 10 ft^2 (0.9 m^2)
  - Level 2: Mid-sized isolated areas 10-30 ft^2 (0.9-2.8 m^2)
  - Level 3: Large isolated areas 30-100 ft^2 (2.8-9 m^2)
  - Level 4: Extensive contamination > 100 ft^2 (9 m^2)
  - Level 5: Remediation of HVAC systems

Russian Federation (Sidorov, 1991)
Standard limits are set for some species based on allergenicity in animal models.

- *Aspergillus terrus* = 300 cells/m^3
- *Candida* Scotty yeast = 10^3 cells/m^3

WHO (1997)
Based on literature review. Cites National Health and Welfare Canada guidelines.

OSHA (Rao et al., 1996)
Derived by a review of the literature and from personal experience.
1000 CFU/m^3, 10^6 fungi/g of dust and 10^5 fungi/mL of stagnant water are levels that are indicative of contamination

Based on their own experiments and experience, some scientists have presented their own guidelines for fungal levels:

- Derived from a review of the literature, Burge et al. (1987) state that indoor levels of mould should be less than one-third of outdoor levels where outdoor air is the only source and should be qualitatively similar.

- Miller et al. (1988) sampled 50 Canadian homes, the majority for which the owners had complained about respiratory of allergic problems, with a Reuter Centrifugal Sampler (RCS) and Rose Bengal (RB) agar. Fungal levels for this study ranged from 0-3,120 CFU/m^3. The following indoor air guidelines were presented: a) fungi such as *S. chartarum* are unacceptable, b) >50 CFU/m^3 of any species requires further investigation, c) >150 CFU/m^3 is acceptable if there is a mixture of species, other than pathogens, and d) up to 300 CFU/m^3 are acceptable if the species are mainly *Cladosporium*.

- In a study designed to determine typical concentrations of fungi in an office environment, Morey and Jenkins (1989) found that concentrations of indoor fungi are typically 10-15% of those found outdoors.

- Over a three year period, Yang et al., (1993) collected over 2000 fungal samples with an Andersen N-6 sampler on 2% MEA. With this baseline data, they recommend a 200 CFU/m^3 guideline for fungal bioaerosols because 75% of the indoor samples yielded fungal concentrations less than 178 CFU/m^3.

- A study was conducted in 631 schools in which 4,800 fungal samples were obtained using a RCS and RB agar (Rand, 1998). For non-contaminated schools, the proportion of soil dwelling genus (*Aspergillus, Penicllium)* to phylloplane genus (*Alternaria, Aurobasidium, Cladosporium*, non-sporulating, *Ulocladium*) is low.

- Godish (2001) reported that nonresidential buildings that are mechanically ventilated should have a fungal concentration of less than 300 CFU/m^3, and that moderately infested buildings would have between 1,000-3,000 CFU/m^3.

2.6 Mycological Air Sampling

Mycological air sampling is divided into two techniques. One is based on the growth and germination of viable mould particles, and the other is based on the capture and the observation of both living and dead organisms. The former technique is achieved through air sampling, whereas the latter method involves obtaining bulk, tape, and swab samples (ACGIH, 1995). Another method to test for the presence of fungal contamination is to make indirect measurements of potential fungal markers such as VOCs and mycotoxins. In general, there is not one single technique that provides the complete picture of the presence of mould in a building (Miller, 1992).

Because there are many difficulties associated with collecting spores that have different shapes, sizes, and masses (Minister of National Health and Welfare, 1995), sampling methods tend to underestimate actual spore concentrations (Burge, 1986), and are thus described as semi-quantitative. The values obtained by sampling are influenced by collection efficiency, proper usage of the sampler, particle size distribution of the spores, spatial distribution of spores in the environment (Yoshizawa et al., 1993), and the type of culture media (AIHA, 1996). Due to the inappropriateness of establishing a single comprehensive set of criteria to cover all bioaerosols and bioaersol samplers, no formal performance criteria have yet been proposed (Macher et al., 1995).

One of the simplest techniques available to sample for mould is the gravity settling culture (GSC) plate. A petri dish filled with agar medium is left open to the air for approximately one hour, during which time particles settle via gravity. Studies indicate that GSC plates are biased as they mostly capture large particles that settle according to their density (FPCEOH, 1995; Sayer et al., 1969).

There are five types of viable air samplers which can be categorized into those that impact a stream of air onto a fungal medium (slit-to-agar, multiple-hole and centrifugal samplers), those that trap propagules from an air stream in a viscous fluid (liquid impingers), and those that trap propagules on a membrane filter. Common features to all bioaerosol samplers include an air inlet, a particle collector, a flowmeter, and a flow rate controller (Macher et al., 1995). Appendix 1 provides a description of many microbiological air samplers.

Many studies have attempted to compare the sampling efficiencies of bioaerosol samplers, yielding varied and sometimes conflicting conclusions. For example, one study conducted by Smid et al. (1989) investigated the Andersen N-6 sampler, a slit sampler, and a RCS and found that they were comparable in determining the number of CFU. Another study by Mehta et al. (1996) concluded that the Burkard portable air sampler and the Andersen two-stage sampler were comparable, but that the total CFU collected by the RCS Plus was significantly less than the other two samplers. According to Burge et al. (1987), the slit-to-agar samplers most efficiently collect viable bioaersols. Another study completely contradicted this by stating that the rate at which airborne microorganisms are recovered with the RCS is significantly higher in terms of CFU/m^3 of air than that of the slit-to-agar or the liquid impingers (Delmore and Thompson, 1980). The FPCEOH (1995) indicates that these comparison studies should be interpreted with caution as they include uncontrolled variables such as failing to standardize sample duration and volume.

As the section above illustrates, there are a plethora of microbiological sampling devices and without some type of standard, it is left to the discretion of the investigators to choose an air sampler that best suits their needs. Devices that operate at fairly high flow rates but are quiet and unobtrusive are recommended for sampling in residences, offices, and healthcare settings. Those that are battery operated offer an added convenience (Macher et al., 1995). ACGIH (1989) even goes so far as to say

that air sampling should only be used as a last resort in identifying the source of bioaerosol contaminant, and that only in research situations should it be used.

Besides air sampling, many IAQ studies rely upon both bulk and surface sampling to detect the presence of settled mycoflora. For bulk sampling, small pieces of material such as carpets and wallboards are collected and sent to the laboratory for analysis where tests determine whether the fungus has colonized the material (Martyny et al., 1999). Surface sampling using swabs or tape also play a useful role in sampling visible mould growth. Surface sampling is preferred over bulk sampling because it is a non-distructive technique (Martyny et al., 1999). Collection of adhesive tape samples involves gently placing a three-inch strip of clear tape onto suspected mould growth, carefully removing it, and then placing it face down onto wax paper or a glass slide (Martyny et al., 1999). Investigators are also encouraged to submit a blank tape sample for comparison under a microscope. This tape-lift method is not quantitative but it can provide information on the types of species present (Martyny et al., 1999). Taking a swab sample involves wiping a wet sterile swab to a suspected mould surface. The swab is then placed into a sterile container and then shipped to the laboratory for analysis.

As previously indicated, there are also indirect ways of measuring fungal presence. These chemical analyses are gaining popularity because they give faster measurements (Nielsen, 2001) however they often require much larger air volumes to account for the methods' low sensitivities (Burge and Otten, 1999). Bush and Portnoy (2001) suggest a variety of chemical markers that can be used to detect fungal presence and these are described in Table 7. These metabolites can be measured using GC-MS, High Performance Liquid Chromatography (HPLC), and capillary electrophoresis (Nielsen, 2001).

Table 7. Chemical Markers of Mould Growth

Marker	Description
Ergosterol	• Primary cell membrane sterol of most fungi • The amount produced depends on the fungi's surface area and growth conditions • Levels correlate well with total spore count though are not helpful indicators of individual species • ~3.2 μg ergosterol/mg spore (AIHA, 1996)
β-1,3-glucans	• Glucose polymer found in filamentous fungal cell walls • Both living and dead spores are detected
VOCs	• Metabolites such as alchohols, aldehydes, and ketones • Not specific for fungi: they are produced both naturally and anthropogenically • Sum of 13-20 VOCs can be used to estimate total fungal VOC levels (AIHA, 1996)
Extracellular Polysaccarides	• Carbohydrates secreted or shed during fungal growth

2.7 Air Sampling Media

There are many types of fungal media upon which to collect air samples. A desirable medium will support the growth of as many fungal species as possible, and inhibit very few (Burge, 1990). There are several problems inherent to viable sampling and the type of agar medium used. Firstly, some organisms have extremely specialized growth requirements and will not germinate on all artificial culture media (Burge and Solomon, 1987). For example, *Stachybotrys* species are not viable on agar medium specific for xerophillic mould, and thus a medium enhancing its growth, one with high water activity, low nutrient concentration and some cellulose content, should be used (AIHA, 1996). Secondly, some fungal species produce powerful antibiotics that diffuse into the culture medium and prevent the growth of other fungi within a one centimetre radius (Burge, 1985). Thirdly, many airborne spores are not living at time of sampling (Burge, 1985), and thus will not grow on the media during

the incubation period, leading to a false negative or low result. And lastly, overcrowding of the medium may also drastically reduce quantitative recoveries (Burge and Solomon, 1987).

The four most commonly used agar media for fungal air sampling are RB, MEA, Dichloran 18% Glycerol (DG-18), and Sabouraud Dextrose Agar (SDA). RB agar, which is recommended by National Institute for Occupational Safety and Health (NIOSH) for sampling indoor occupational environments (Smid et al., 1989), restricts radial growth of hyphae and minimizes confounding effects of spreading and overgrowth (Morring et al., 1983). It contains streptomycin which inhibits bacterial growth, and also restricts the growth of *Rhizopus* and *Mucor* (Biotest Inc., 2001). Literature also indicates that RB agar is sensitive to direct sunlight and should be shielded when sampling outdoors (Smid et al., 1989; Morring et al., 1983; Burge et al., 1977). MEA, which is recommended by ACGIH committee for indoor environments (Smid et al., 1989), promotes the growth of saprotrophic fungi (Biotest Inc., 2001), but without an antibiotic ingredient, also permits high numbers of bacteria to grow (Burge et al., 1977). AIHA (1996) reports that DG-18 promotes the growth of xerophillic saprotrophic fungi, and that it also restricts growth of fast growing genera, like *Rhizopus* and *Mucor* (Smid et al., 1989). And lastly, SDA, which is rich in carbohydrate, is not recommended due to a substantial quantity of glucose which favours rapidly growing species and promotes vegetative growth, thus delaying the production of spores for identification (AIHA, 1996).

Table 8 lists the ingredients in the four most common agar media used to grow mould samples for air sampling. Other fungal media include oxytetracycline glucose yeast extract agar (Verhoeff et al., 1990), Littman Oxgall agar (Morring et al., 1983), V-8 (Larsen, 1981; Burge et al., 1977), and inhibitor mould agar (Morring et al., 1983). New batches of agar for sampling are required on a continual basis as they have a shelf-life of three months (Biotest Inc., 2001).

Table 8. Ingredients for Preparing Agar Media with 1 L of Deionized Water

Medium	Ingredients
RB[a]	10 g glucose, 6 g peptone, 3.7 g KH_2PO_4, 9.3 g K_2HPO_4, 0.5 g $MgSO_4 7H_2O$, 40 mg streptomycin, 50 mg rose bengal, 16 g agar
MEA[b]	20 g malt extract, 20 g dextrose, 1 g peptone, 15 g agar
DG-18[b]	10 g glucose, 5 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4 \bullet 7H_2O$, 0.1 g chloramphenicol, 15 g agar, 220 g glycerol, 2 mg dichloran
SDA[a]	40 g glucose, 10 g peptone, 15 g agar

[a] Malloch, 1981
[b] Burge and Otten, 1999

A review of literature did not produce consensus on which medium was best to use, and there was even some conflicting information. For example, RB agar is commonly used for broad spectrum aerobiological sampling because of its antibacterial ingredients (Biotest Inc., 2001; Burge et al., 1977). Burge et al., (1977) however state that an inherent risk to using RB agar is that it sporadically suppresses fungal growth on parts of the medium. Another example of conflicting results includes two studies that tested the same three agar media: DG-18, RB and MEA. Smid et al. (1989) found that each medium performed equally, whereas Verhoeff et al., (1990) found that the highest yield was obtained with DG-18, and that MEA and RB were comparable.

2.8 Recommended Techniques for Mould Sampling

In brief, there is neither one infallible method nor any consensus among scientists on how to sample for mould. A survey of the literature yields significantly different experimental methods and procedures. At the very least, WHO (1988) states that any sampling strategy must address the spatial and temporal distribution of the biological agents, and be accompanied by detailed information about the site and the circumstances of collection. There are two essential books (ACGIH, 1989 and AIHA, 1996) that provide very useful information to the occupational health or the indoor air

investigator. The sections below highlight important techniques that are required for successful mould sampling.

Despite the methodological variations among references, there are several concepts that are common to many studies. Most of the research emphasizes the importance of using several sampling techniques to detect mould contamination (AIHA, 1996; Burge, 1990; Jarvis, 1990; Kozak et al., 1980). These methods include using volumetric air samplers to capture viable spores, using clear Scotch tape imprints, bulk and swab sampling to obtain both viable and non-viable spore identification, and also completing a detailed inspection of the space being sampled (also know as a walk-through).

Many sources also suggest following certain quality assurance and quality control (QA/QC) procedures. First, all samplers should be calibrated to manufacturers' flow-rate specifications before use (Burge et al., 1987). Second, ACGIH (1989) advises conducting sequential back-to-back duplicate samples, which measure the precision of the collection method. Third, field and laboratory blanks should be undertaken in order to detect contamination during sample collection and during sample analysis respectively (Macher, 1999). If possible, a controlled experiment involving a known amount of mould should be conducted to determine the accuracy of the method.

Previous research highlights the importance of sampling during normal occupant activity because human movement affects suspension and sedimentation of fungal spores (Miller, 1992; Health and Welfare Canada, 1987). Buttner and Stetzenbach (1993) evaluated the effects of human activity on air sampling, and determined that disturbance by walking or vacuuming in rooms with high spore loads resulted in significantly higher concentrations of airborne spores than in undisturbed conditions. References also recommend collecting as many samples as possible because bioaerosol concentrations vary throughout space and time (Macher et al.,

1995). Studies have even shown that within the same room, fungal levels may differ (Rao et al., 1996). Because grab samples may not adequately represent average or peak exposures (Dales et al., 1991), it is suggested that sampling occur at different times during the day, and on different days (Burge et al., 1987). Ideally, samples should be taken at several locations throughout the area, including air outlets, mechanical rooms, supply plenums, outdoor air intakes and at occupant breathing level (Minister of National Health and Welfare, 1995). During a walk-through of a building, it is recommended that the HVAC systems be looked at as they are considered to be one of the major indoor reservoirs and amplification sites for fungal contamination (ACGIH, 1989).

2.9 Statistical Interpretation of Fungal Results

Once data have been collected, correct analysis is essential. ACGIH (1989) highly recommends the consultation of a statistician to verify data analysis methods. Research indicates that bioaerosol data rarely, if ever, follow a normal population distribution and require either log transformation or most likely, the use of nonparametric tests (ACGIH, 1989; Burge et al., 1987; Burge and Solomon, 1987). Nonparametric tests, which make no assumption about variability or form of the population distribution, are computationally simple, not as flexible with respect to hypothesis, not as powerful, and deal with ranks rather than values of observations (Zolman, 1993).

The majority of bioaerosol counts fall between zero and the mean therefore are positively skewed (Macher et al., 1995; ACGIH, 1989). Neither the mean nor the standard deviation will be good estimates of data that are positively skewed with a few high data points (Helsel, 1990). Alternative measures of central value and variability for skewed data are percentile parameters such as the median and the interquartile range (IQR). The median has 50% of the values above it and 50% below it. The median is not strongly affected by a few high or low values. IQR is the 75th

percentile minus the 25th percentile and thus is the range of the central 50% of the data (Helsel, 1990).

Some reports state that CFU levels in buildings are unimportant in the context of evaluating whether a building is normal or suffering from bioamplification or contamination problems (Rand, 1988). Far more important and useful is an evaluation of the species assemblages associated with building samples (Rand, 1998) and they should be tabulated accordingly. Likewise, because there are many biases in bioaerosol sampling, rather than listing concentration measurements for each individual taxa, investigators may list each agent as a percentage of the total for that sample (Macher, 1999).

Research indicates that outdoor air provides a reference point for the determination of whether or not certain taxa are being amplified (Burge et al., 1987). An informal comparison between indoor and outdoor fungal numbers is often sufficient to demonstrate the obvious differences in biodiversity when an indoor environment is heavily contaminated (AIHA, 1996). If the difference is not clear-cut, a statistical approach such as Spearman's nonparametric rank correlation might be more appropriate (ACGIH, 1989; AIHA, 1996). During the wintertime in sub-arctic climates, some scientists do not take outdoor air when snow covers the ground, because they assume that outdoor counts would be extremely low. Consequently, the contribution of outdoor spores to indoor air fungal numbers is considered negligible (D'Amato and Spieksma, 1995; Hyvärinen et al., 1993; Lehtonen and Reponen, 1993; Burge, 1985).

All cited literature within this and other sections of this report have been referenced in Section 6.0. Should the reader require additional valuable reference material, these have been included in Appendix 2.

3.0 EXPERIMENTAL METHODS AND PROCEDURES

3.1 Health Review Ethics Board

The purpose of the University of Alberta Health Review Ethics Board (HREB) is to scrutinize research proposals to ensure that the best interest of the subjects participating in the study are being met (ie. respecting their will, privacy and confidentiality). A full HREB Panel B review was triggered because the study would involve contacting human subjects including students, staff, and employees (HREB, 2001). The submission to the HREB, which included both general and specific details of the project, can be found in Appendix 3. Ultimately, the HREB committee stated that the nature of the study was not one that required their approval, and that the investigation could proceed.

3.2 Experimental Design

To determine baseline numbers of moulds in schools within the CHA region and to determine whether they are considered to be problematic, an ideal scheme would be to observe every individual school in the population (Barnett, 1974; Yates, 1971). But due to limitations of resources such as time and money, it was not possible to do this. Consequently, a carefully constructed experimental design was devised, that was logistically acceptable, minimized sampling variations, and maximized sample size.

3.2.1 Sample Size

There are numerous methods used to determine the sample size of an experiment. Typically, sample size is calculated based on cost, precision of the instrument or the measurement, and prior sample variance (Cochran, 1977). When prior information is not available, pilot and pre-tests are often used to help gauge the total number of achievable samples by estimating the resources required to make an individual observation. Because the distribution of means from a non-normal population will tend towards normality as the sample size increases, a minimum of 30 schools was targeted for sampling (Zar, 1974). As indicated in Section 2.9, fungal

data are non-normal and should be analyzed using nonparametric tests that do not make assumptions about the form of the population distribution. Other data however, such as ventilation and comfort parameters were collected during the fieldwork and were amenable to parametric tests. In addition to these statistically-based guidelines to determine sample size, consideration was also given to proportionally allocating schools from each district. A total population of 217 schools (listed in Appendix 4) was broken down as follows: 189 EPS (87%), 20 EIPS (9%), and 8 EICS (4%).

In the absence of prior knowledge, professional statistical assistance was sought in order to find an optimal sample size. Dr. Jhangri (2001), Professor of Statistics in the Department of Public Health Sciences at the University of Alberta, indicated that a 20% rule of thumb, which would result in the sampling of 43 schools, would adequately represent the total population of 217 schools. The distribution of the 43 schools among the three districts was then proportionally allocated, resulting in 37 EPS, 4 EIPS, and 2 EICS. In order to decrease the time and the expense incurred by the EPS board however, EIPS and EICS both agreed to increase their sample size to 7 and 4 respectively. Ultimately, 32 EPS, 7 EIPS, and 4 EICS were sampled or 17%, 35%, and 50% respectively.

3.2.2 Stratification

Stratification is a technique in which prior knowledge about a population is used to form independently sampled groups called "strata" (EPA, 2000a; Satin and Shastry, 1993). Research indicates that stratification may result in an increase in precision (Stuart, 1984; Cochran, 1977) as well as tend to exclude the more extreme possibilities that are included under simple random sampling (Stuart, 1984). The effect of the gain in precision depends on the variation among stratum averages. The greater the variability among the stratum averages, the greater the gain in precision (Stuart, 1984). Proportional allocation is used to allocate samples to the strata in a manner such that the proportion of the total sampling units allotted to a stratum is the

same as the proportion of sampling units in the population that are classified in that stratum (EPA, 2000a).

A pre-screening survey tool was designed to assist with school selection and consisted of ten questions that were answered by facility personnel from each school board (Appendix 5). For ease of reference during the proceeding discussion, the 10 questions are included in Figure 3.

1.	What category does the school fall into?
	K-6: ☐ K-9: ☐ K-12: ☐ 7-9: ☐ 7-12: ☐ 9-12: ☐
2.	What is school utilization rate (%) based on the 2001 Alberta Infrastructure guideline?
3.	What type of foundation(s) are found in school excluding portables/pods?
	Basement: ☐ Slab-On-Grade: ☐ Crawl Space: ☐
4.	What year did the initial construction of school take place? _____
5.	Are portables and/or pods present? (Y/N)
6.	What type of system(s) are used to heat school?
	Forced Air: ☐ Hot Water/Steam: ☐ Other: ☐
7.	Has school ever had a modernization and/or a heating and ventilation system upgrade? (Y/N)
8.	In the past 5 years, has the school had a history of water damage? (Y/N)
9.	In the past 5 years, has the school had an investigation of IAQ concerns? (Y/N)
10.	In the past 5 years, has the school ever had to take actions to resolve fungal contamination concerns? (Y/N)

Figure 3. Abridged Pre-Screening Survey

Using the information obtained from the pre-screening survey, methods for stratification were decided upon with the assistance of a statistician (Prasad, 2001). It was recommended that there be no more than three strata because the population size was not large enough to support such narrowing. From pre-screening results (Appendix 6), several suggestions were made on how to best stratify into two levels.

It was first suggested that IAQ concerns (Q.9, Figure 3) be used as one level of stratification, however this idea was discarded due to the subjective nature of the question. As indicated in Section 2.2.3, there is a difference in fungal numbers between rural and urban schools and for this reason school location was also considered as a stratum level. Stratifying in this manner was rejected however because there were only nine rural schools in the total population of 217. It was then proposed that utilization rate (Q.2, Figure 3) be a stratum level. Based on cursory analysis of the pre-screening data, a histogram of the utilization rate (Figure 4) revealed a continuous set of normally distributed results with a mean of 73.6% and a range of 23.9%-169%. Research indicates that stratification should only be used when it is expected that the dividing features will actually be different (Stuart, 1984), and therefore the utilization rate would best be captured by random sampling.

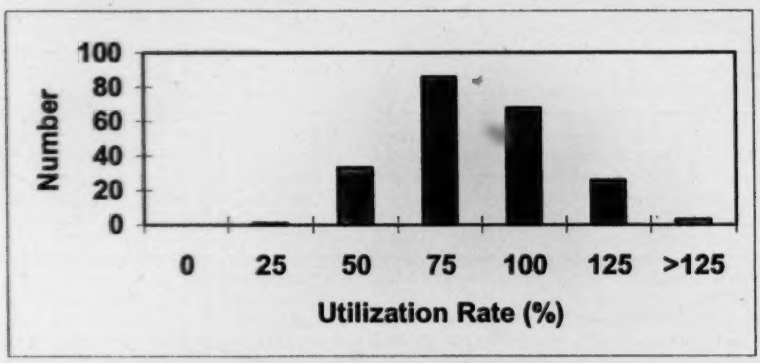


Figure 4. School Utilization Rate for Schools Within the Population

The presence of portable and pod building structures (Q.5, Figure 3) was also considered as a stratum, but was eliminated because the differences between permanent, portable, and pod structures would be captured by a subset, which will be described in Section 3.2.6.3. Grade level (Q.1, Figure 3) was also considered to be one of the strata levels, but was discarded because there were too few upper level schools from which to choose, thus needlessly narrowing the sample size in each stratum. Ultimately, the sample design was stratified into two levels: geography and history of water damage and/or fungal investigation, and is discussed further below.

3.2.2.1 Geographic Location, Water Damage and/or Fungal Investigation

Based primarily on geographical location, Alberta Infrastructure has divided EPS into nine sectors (Wilson, 2001). In order to increase the number of schools within each sector, these Edmonton administrative boundaries were collapsed into five geographic zones that were created based on geographical proximity. With EIPS and EICS combined into another sector, a total of six zones were established for this study (Figure 5 on the following page).

Based on pre-screening results, within the past five years, 39 schools had reported a history of water damage and 34 schools had taken action against fungi. Combining these two parameters, a history of water damage and/or fungal investigation provided enough schools in this combined category for a second stratum. Because there were seven schools that had both a history of water damage and had completed fungal investigations, the total number of schools within both categories was 66.

3.2.3 Random Sampling

After having stratified to two levels, random sampling without replacement was performed within each stratum. Choosing schools at random ensured that each member of the total population had an equal and independent chance of being selected (Satin and Shastry, 1993). Each school was assigned a unique number within their stratum and then a set of numbers was drawn from a random number chart whose digits from 00-99 had an equal frequency of appearing (Zar, 1974). Once the first random number was blindly selected, the decision was made to proceed down the column (Satin and Shastry, 1993) to obtain the required number of schools per stratum. If the same random number appeared more than once, this value was disregarded the second time (sampling without replacement). Table 9 contains a list of the 43 randomly selected schools.

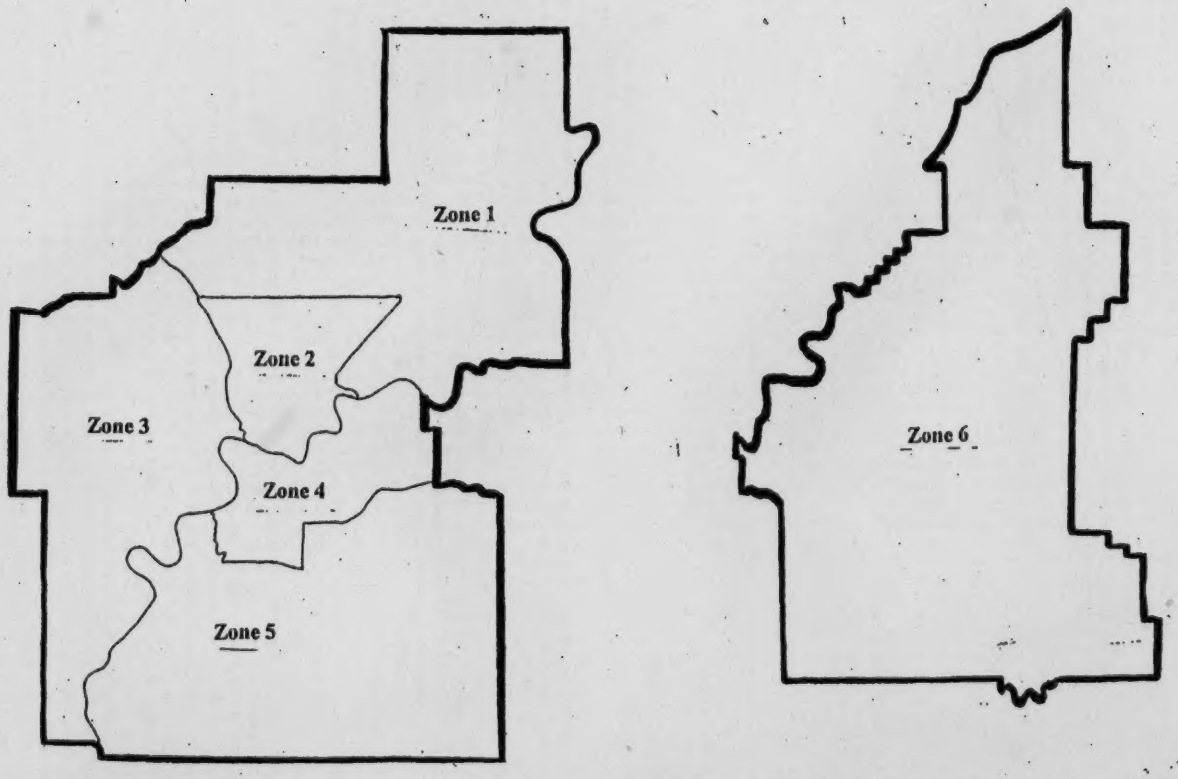


Figure 5. Adjusted Alberta Infrastructure Geographical Zones Used for First Level of Stratification (after Wilson, 2001)

Table 9. Randomly Selected Schools Presented by District

EPS			EIPS	EICS
Belgravia	John. D. Bracco	Ottewell	Cloverbar	Arch Bishop Jordan
Clara Tyner	Julia Kiniski	Rio Terrace	Colchester	Holy Redeemer
Coronation	Lauderdale	Riverbend	FR Haythorne	Jean Vanier
Crestwood	Laurier Heights	Ross Sheppard	Pine Street	Our Lady of
Duggan	Lorelei	Sakaw	Salisbury	Perpetual Help
Eastglen	McCauley	Satoo	Westboro	
Eastwood	McKernan	Sifton	Woodbridge	
Elmwood	McNally	Tipaskan	Farms	
Fraser	Mee-Yah-Noh	Wellington		
Glendale	Menisa	Windsor Park		
Harry Ainlay	Newton			

3.2.4 School Recruitment

After the schools had been randomly selected within the confines of the experimental design, packages consisting of an introductory letter to school principals, a newsletter for staff and parents, a response form, and an investigator conduct protocol, (Appendices 7 through 10) were distributed to schools via district representatives. In the event that schools declined to participate, other randomly selected schools had been placed on a waiting list and were to be contacted by the school board representative if necessary. In all cases but two, principals agreed to participate in the IAQ study by faxing a signed copy of the response form back to the University. The next step involved contacting each school to schedule a visit and to let them know which rooms would be sampled. It was during this time that school contacts could ask any questions that they might have regarding investigator presence in the school.

3.2.5 Number of rooms

Facility personnel completed several surveys (described in Section 3.4) prior to sampling in order to provide investigators with advanced knowledge about each school. This prior knowledge was originally intended to help select rooms according to their known characteristics. For example, ACGIH (1989) suggests sampling one control area and several contaminated locations within each building. Looking at results from the surveys however, it was not possible to determine with certainty

which rooms could be considered control locations and which rooms could be considered to have uncorrected water damage problems. This was in part due to the fact that the reference period for the questions asked in the surveys were extended over the past five years, and may not be reflective of actual circumstances. In the end, all rooms were assumed to not have any distinguishing features, and were randomly selected.

Similar to choosing a sample size, determining the number of rooms to be sampled per school to obtain representative data required the help of a statistician. Shannon's Sampling Theorem, which provides a perfect air sampling strategy, states that more than 250,000 samples per cubic meter per hour are required to obtain accurate information about airborne concentrations in the room as a function of space and time (ACGIH, 2001). This is an extremely unreasonable and impossible sampling strategy. Since this was a baseline study, it was seen as more important to include more schools in the study, with lower frequency of sampling within the schools (Probert, 2001). As a result, between three to five rooms were visited at each school, for a total of 202 rooms. As will be described in Section 3.2.6.1, two sampling rounds were completed. During the first round, three rooms per school were sampled, whereas in the second round, five classrooms were visited. The major reason for the increase in the number of rooms that were completed per school in the second round was due to increased investigator proficiency while conducting experiments in the field.

3.2.6 Subsets

Due to experimental limitations including cost and time, a common practice to gain a more thorough understanding of a particular research parameter is through the use of subsets or sub-samples (Stuart, 1984; Cochran, 1977). The results obtained in the subsets are then transposed onto the sample size, and then inferences are made to the total population. The subsets that were taken during the study, and which are described in this section, deal specifically with sample and analysis of fungal

contaminants (Figure 6). All schools participating in the subsets were randomly selected. In some instances however, depending on the nature of the subset, selected schools may have had to meet certain requirements (ie. having pods), and if these were not met, then the school was not chosen for the subset.

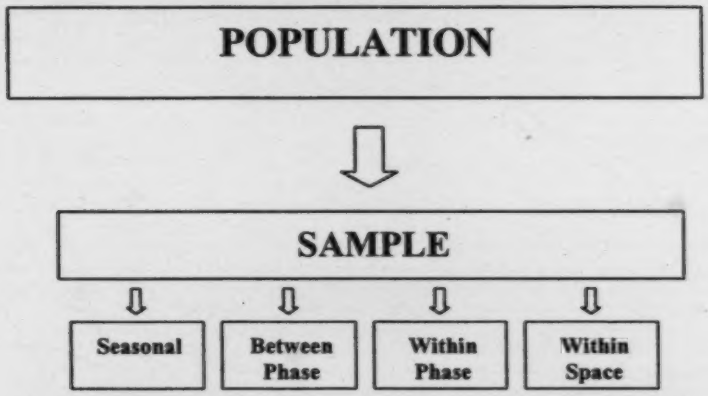


Figure 6. Subset Layers (see Sections 3.2.6.1-3.2.6.3 for explanation)

3.2.6.1 Seasonality

Seasons are regulated by the amount of solar energy received at the earth's surface, and are primarily determined by the angle and the latitude at which sunlight strikes the surface (Ahearns, 1994). Seasons in the Northern Hemisphere are defined in two ways (Table 10). Astronomical definitions consider a new season when the earth, revolving around the sun in its elliptical orbit, is either furthest away from the sun (solstice) or closest to the sun (equinox). Meteorological definitions consider that seasons are based on the warmest and coolest portions of the year, followed by the transitions into each extreme.

Table 10. Astronomical and Meteorological Definitions of Seasons

Season	Astronomical	Meteorological
Spring	March 20 - June 20	March, April, May
Summer	June 21 - Sept. 21	June, July, Aug.
Fall	Sept. 22 - Dec. 20	Sept., Oct., Nov.
Winter	Dec. 21 - March 19	Dec., Jan., Feb.

Research indicates that indoor fungal contamination levels are dependent on season and that they are usually highest in the fall when outdoor concentrations are high due to decay on fallen leaves (Rand, 1998). To adequately interpret the results obtained in this study, it was necessary to account for seasonal differences by sampling in two separate seasons or rounds. Due to the timing of the experiment, the fall and winter seasons were examined. Upon consultation with a statistician (Jhangri, 2001), a subset sample size of ten was judged as adequate to capture seasonal variation. The number of schools chosen from each school district was determined on a proportional basis, resulting in the following breakdown: 8 EPS, 1 EICS, and 1 EIPS. These ten schools were visited once in the fall (round one) and then again in the winter (round two). Resampling occurred at approximately the same time during the day and at the same location within each room.

Fall sampling, which was completed within both the meteorological and astronomical definitions of fall, began on October 10th, 2001 and ceased on November 14th, 2001. Winter sampling began on January 21st, 2002 and finished March 21st, 2002, and was interrupted for 2.5 weeks (February 7 - February 22nd) due to a teacher strike. Although the winter sampling ceased two days past the astrological beginning of spring, cold weather kept temperatures low and comparable to winter climate.

3.2.6.2 Within Space

As indicated in Section 2.8, scientists have discovered that fungal numbers vary within space. These variations are due to non-uniformly mixed air within the room: there are air currents, stagnant air near walls, and short circuiting of some ventilation systems. In order to observe these differences a statistician recommended that at least nine of the 43 schools (or 20%) be chosen to participate in this subset (Jhangri, 2001). The air sampler was placed at three different locations within the same room in nine randomly selected schools within round one. This procedure added an extra 25 minutes of sampling time per classroom.

3.2.6.3 Within and Between Phase

There are three types of building structures common to all schools in this study: permanent buildings, portables, and pods. A portable is defined as a temporary collapsible classroom whereas pods consist of a cluster of classrooms. Both portables and pods were not part of the original main building structure. The first phase of a school always consists of a permanent building structure. After a number of years, if the school required expansion, the added-on rooms would be considered phase two. If a portable was later constructed, this would be considered phase three, and so on until all major construction and renovations had been included. As can be appreciated, if the original school structure was built in the early 1960's, and an expansion did not occur until the late 1970's, the construction material, the classroom design and the ventilation components servicing the new rooms would likely be quite different. Because the majority of schools have more than one phase, it was important to determine the variability of fungal contaminant levels both within and between phases.

The number of phases contained within each school and their location was obtained through a screening survey (Section 3.4.1.2), which was completed by facility personnel prior to room selection. To investigate variability of fungal numbers occurring between phases, a statistician suggested that a minimum of nine schools that contained all three building structures (permanent, portable, and pod) be sampled (Jhangri, 2001). From the screening survey results however, only one school met this requirement. Thus, investigators were either able to observe differences between permanent and portable structures or able to observe differences between permanent and pod structures. To determine variability of fungal numbers occurring within the same phase of the three building structures, investigators needed to sample at least three rooms within the same phase of the same school. This subset however could not be completed for portables as they usually encompassed one phase and only one room. Again, upon the advice of a statistician, nine schools were randomly selected for the within phase subset (Jhangri, 2001).

3.3 QA/QC

The veracity of the data must always be taken into consideration when performing an experiment. Accuracy is the proximity of the measured value to the true value, and is measured by analysis of standard samples. There are currently no reference mould samples that can be used to determine the accuracy of the RCS Plus method without costly controlled laboratory experiments. Precision is the degree of agreement among replicate analyses of a sample, and is reported as the standard deviation. Due to the drawing motion of the centrifugal sampler, sample constituents flowing past the agar strip are always changing, as adjacent air is drawn towards the instrument to fill the void that is created. In essence, as the sample is being taken, conditions within the room are changing. This should not matter however, if it is assumed that the air within the room is homogenously mixed. In order to estimate the reproducibility of the RCS Plus air sampler results, one sequential duplicate air sample, taken in the same location and position, was obtained in the majority of schools sampled.

Several methods were used to determine whether the agar strips were contaminated during handling, collection, transport or analysis. First, the manufacturer of the agar strips certified that representative material from each lot were tested in QC laboratories for sterility after seven days of incubation at two different temperatures, and inhibition of *Staphylococcus aureus* and *Escherichia coli* (Biotest, 2001). Second, once a week, one agar field blank was submitted to the laboratory for analysis. A field blank was used to estimate contamination that might have occurred during sample collection and laboratory analysis. It was handled in the same manner as a normal sample, however when the field blank was inserted into the sampler, no air was drawn, and it was immediately replaced into its package. Third, to minimize laboratory bias, all samples were submitted to the laboratory with coded labeling so that the mycologists did not know which rooms the samples had come from, or whether the samples were duplicates. Fourth, sampling in a 16 m x 16 m x 8 m "clean room" chamber located at Toxcon Laboratories in Edmonton was completed

to ensure that the sampler was free of mould and that the cleaning techniques employed were sufficient. This chamber was temperature and humidity controlled, and contained minimal particulates as the air circulating within the room passed through a series of HEPA filters. Assuming that this chamber was free of mould contaminants, the RCS Plus instrument was placed in the center the room and was exposed to a humidity of 15% and a constant temperature of 22°C (Section 4.2).

To minimize cross-contamination between agar samples, 70/30 isopropyl alcohol/water wipes were used to clean the instrument, and new sterile gloves were donned by field investigators handling the equipment between samples. In order to disinfect the RCS Plus impeller, it was left to soak in a 70/30 isopropyl alcohol/water solution overnight, and then air dried before use. Using the manufacturer's anemometer, the RCS Plus was calibrated once before each sampling round.

3.4 Field Instruments

3.4.1 Field Surveys

Five surveys were completed during the course of this study, three of which were used primarily for experimental design and planning purposes (pre-screening, screening, and school maintenance history surveys). The remaining two surveys (ventilation and room) were used to capture existing conditions when sampling. If anomalous results were obtained, it would then be possible to review survey data to identify a potential cause. To ensure that the survey contents were easily understood, strategies such as keeping the vocabulary simple, using a glossary to clarify key terms, and keeping the questions short were utilized. In order to capture the more recent changes that had occurred in schools, the reference period for historical information was narrowed to within the last five years.

For maximum consistency, it was recommended that all surveys be completed by the investigators and not by staff, principals, or facility personnel (Prasad, 2001). This suggestion was not possible to achieve however because some information was

only accessible to facility personnel. To minimize responder bias, the same facility person within each district completed all of the surveys of one genre. To minimize responder bias between three the field investigators involved in the study, brief trial runs were conducted to standardize responses. Furthermore, when an element was unclear while in the field, all three investigators were present in each sampling location to confer.

3.4.1.1 Pre-Screening Survey

As described in Section 3.2.2, a ten question pre-screening survey (Appendix 5) was administered to all schools within the population in order to assist with the experimental design process. A facility personnel representative from each school district completed these surveys within three weeks and the results are included in Appendix 6.

3.4.1.2 Screening and School Maintenance History Surveys

Once the experimental design had been decided upon and 43 schools had been randomly selected, screening and school maintenance history surveys were administered. These surveys, which can be found in Appendices 11 and 12 respectively, took approximately 20 min to complete. Facility personnel who completed these two surveys were the same people that had filled-out the pre-screening surveys, thus minimizing responder bias. Once completed, investigators received maps of the schools, the number and location of phases in each school, obtained information on the maintenance schedules of each school, and also gleaned information regarding previous water damage.

3.4.1.3 Ventilation and Room Surveys

With the assistance of a facility person, while at each school, the investigators filled out a ventilation survey (Appendix 13). In order to complete the survey, the system was turned off, and the filters, mixing boxes, heating coils and cooling coils were examined for general cleanliness and problems. The expert help of facility

personnel from each district who were familiar with each system was essential to the successful completion of this survey. Inspection of simple unit ventilators took approximately 20 min, while inspection of central systems took 40 min.

While sampling occurred within each space, the investigators completed a room survey (Appendix 14). This survey was used to obtain general conditions of the space during sampling and for reference purposes. Information such as the presence of carpet, occupant level and activity, visibility of previous water damage or any mould growth, and room volume were obtained.

3.4.2 Field Tests

To increase their familiarity with ventilation systems and with sampling procedures, investigators conducted several field tests prior to school sampling. A tour of an HVAC system and a unit ventilator was carried out at the University of Alberta. With the help of EPS board staff, one school's HVAC system was thoroughly investigated, and subsequently, the ventilation survey was scrutinized to reflect actual circumstances. After having near final drafts of the room and ventilation surveys, the investigators conducted a pilot test in one school. During this test, experimental procedures were refined and operational limitations were determined.

3.4.3 Protocols

Originally completed as part of the HREB application, detailed conduct and sampling protocols (Appendices 10 and 15 respectively) were created for investigator use. The sampling protocol illustrates step-by-step specific procedures used for operation and maintenance of all instruments in the field. Although there was not a standard air sampling method found in the literature, the sampling protocol was compiled using reputable reference materials including ACGIH (1989) and AIHA (1996). A conduct protocol was established and followed so that the principals and staff were aware of investigator activity within their school. This protocol was also

established to give investigators standards for proper behaviour, etiquette and relations in all schools.

3.4.4 Field Procedure

For safety reasons, the equipment was left in one of the Environmental Engineering building staging laboratories at the University of Alberta over night. First thing in the morning, all of the equipment and the field investigators were picked up at the University of Alberta. The investigators arrived at the school at 8:00 am and the equipment was unloaded from the vehicle. As per the conduct protocol, the school's contact person was met at the main office, and then the equipment was placed in an accessible, secure location for the day. Either the contact person or the facility person then showed the investigators to the randomly selected rooms. Following this, the facility person gave the investigators a tour of a ventilation system linked to one of the rooms being sampled, and the ventilation survey was completed. Subsequently, and while classes were in session, investigators sampled the rooms as per the sampling protocol. Often, teachers would ask the investigators to share additional information about the study with the students.

After the rooms had been sampled, an outdoor control sample was taken at least 6 m from the main entrance. After the visit was complete, the agar samples were promptly transported to the Provincial Laboratory of Public Health for Northern Alberta (Edmonton) for analysis.

3.5 Mycological Sampling and Analysis

As indicated in Section 2.6, several methods exist to sample for both living and dead fungal contaminants. The two sections below describe procedures involved with obtaining bulk, surface and air samples, as well as detail analytical procedures used in the mycology laboratory. A detailed step-by-step sampling protocol is also included in Appendix 15.

3.5.1 Bulk and Surface Samples

Investigators were not permitted to obtain bulk samples of fabrics and carpets, as this would constitute destruction of school property. Bulk water samples were taken if a pool of water was observed during ventilation system inspections. These water samples were housed in a sealed sterile container and placed in a cooler for transportation to the laboratory. Tape or swab samples were taken if mould deposits were suspected during inspection of diffusers. Tape samples were placed on wax paper along with a blank, and placed in a ziplock bag that was also kept in the cooler. Swab samples were individually contained in sterile Amies media, and were also placed in a cooler while awaiting transportation to the laboratory. All samples were transported by vehicle to the Provincial Laboratory of Public Health for Northern Alberta in the afternoon of each sampling day.

At the laboratory, homogenous aliquots of bulk water samples were concentrated onto a membrane filter and then cultured on Phytone media and Dichloran Rose Bengal Chloramphenicol (DRBC) agar. Clear tape samples were removed from their wax paper backing, mounted on a glass slide, and then observed under the microscope for identification. Bulk swabs were analyzed by touching the swab to a petri dish and cultivating the residues on Phytone media and DRBC agar for 5-7 days at 25°C until identification of fungal spores could occur (Sand, 2001).

3.5.2 Air Samples

As Section 2.6 illustrates, there is an abundance of mycological air sampling instruments. The RCS Plus (Biotest Diagnostics Corporation, Denville, NJ) shown below in Figure 7 was chosen for use in this study because it is a lightweight, portable instrument, and also because scientists have used it with success (Benbough et al., 1993). RB agar was used in conjunction with the RCS Plus.

Figure 7. Diagram of the RCS Plus Sampler (Courtesy of Biotest Diagnostics Corporation, Denville, NJ)

A strip of agar with a surface area of 34 cm^2 is fixed to the outer casing of a rotating cylinder so that the medium faces inwards. Air is then drawn into the RCS Plus at 100 L/min and approximately half of the air is conveyed through slits and past the layer of agar while the other half flows between the protection hood and the outer casing of the rotating cylinder. Although the exhaust outlets and the suction area are situated relatively close together, studies conducted at the Fraunhofer Institute of Toxicology and Aerosol Research (1991) indicated that suction of air into the RCS Plus occurs without spin or whirling, and that recirculation of the flow does not occur. The manufacturer recommends operating the RCS Plus in a vertical position in order to obtain maximum air velocity (Biotest Inc., 2001).

Literature recommends that the RCS Plus air samples be taken at the center of the room, away from non-mixed air near walls or near air leaking in from windows (ACGIH, 1989). It is also recommended that the instrument be placed at the breathing height of the occupants in order to represent the height at which breathing occurs (ACGIH, 1989; Waegemaekers et al., 1989). Once the agar strips, which had been stored in a chilled cooler, were received at the laboratory, they were promptly incubated for 7-10 days at 25°C. The colony forming units were counted and Equation 6 was used to calculate CFU/m^3. Assuming 100% recovery, the detection

limit for a 6 min, 300 L RCS Plus sample was reported as 3 CFU/m^3 (Sand, 2001), which was based on a detection of 1 colony per 300 L.

$$CFU/m^3 = (\text{number of CFU} \times 1000\ L/m^3) / \text{sample size (L)} \qquad [6]$$

After colony enumeration, each colony was mounted on a slide and identified microscopically. Colony morphology included the colours of the surface and bottom of the colony, the texture of the surface (powdery, granular, woolly, cottony, or velvety), the topography (elevation, folding, margins), and the rate of growth (Sand, 2001). If, due to crowding, some colonies were too small to identify, they were removed and placed on Phytone agar and then re-incubated.

Some moulds were identified to the genus level whereas others were identified to the species level. This was due to cost and ease of identification. Organisms like *Cladosporium* are normal leaf flora and it would not be worth the time and cost to identify to the species level. As indicated in Section 2.3 however, *Aspergillus* speciation is relevant to IAQ and is usually fairly easy to complete (Sand, 2001). *Penicillium* species are also relevant however the laboratory does not have much experience at identifying these to species level (Sand, 2001).

3.6 IAQ Advisory

An IAQ advisory form was designed for immediate reporting of unacceptable results or findings observed while sampling. If any of the items listed in the advisory (Appendix 16), were observed (including large amounts of visible mould growth while at the school) then an IAQ advisory form would immediately be faxed to the relevant school board representative. The representative would then make arrangements to conduct a follow-up investigation and/or to remediate the situation.

4.0 RESULTS AND DISCUSSION

4.1 Sampling

The study was well received by principals, staff and students of all 43 schools. Occupants exhibited a variety of responses to investigator presence, including curiosity and sometimes concern, but were always welcoming. Some teachers took the opportunity to make the visit to their classroom into a brief lesson about science and post secondary education and encouraged their class to ask questions. Teachers were also very willing to share their experiences with respect to IAQ within their classroom.

Although it is recommended that the RCS Plus air samples be taken in the center of each room, it was not possible to do this for all classrooms without minimal disruption. Given this limitation, and depending on the congestion and the layout of the room, the RCS Plus was usually set up at the back of the classroom approximately 2-3 m away from the wall. When possible, the RCS Plus was set on a tripod at 1 m off the ground within student breathing height.

Over both sampling rounds, two Edmonton Public Schools were issued IAQ advisories. One school, which had visible mould growth in its boiler room that did not exceed 1 ft^2 (0.09 m^2), was potentially of concern due to the moist and hot environment. In another school, investigators noticed a musty odour in one of the rooms and subsequently issued an IAQ advisory. Once enumeration had been completed on the agar strip for this room however, a low fungal count was observed. There were also six schools (three EPS, one EIPS, and two EICS) in which *Penicillium* levels on the agar strips were found to exceed the 1995 Minister of National Health and Welfare Canada (herein Health Canada) guideline of 50 CFU/m^3. In all instances described above, school district and CHA personnel immediately completed further investigation. In summary, 5% of the 43 schools sampled were issued IAQ advisories for mould-related concerns, and an additional 14% were observed to have greater than 50 CFU/m^3 of *Pencillium* species present.

4.2 QA/QC

A total of six field blanks were performed during both rounds of sampling. On five occasions, the mycological analysis of the agar strips revealed that mould levels were below detection limit (BDL). On one agar strip, 3 CFU/m^3 of non-sporulating species were observed. This could have been as a result of cross-contamination from a previous sample due to insufficient cleaning with the isopropyl alcohol wipes. The fact that five out of six field blanks had below detection levels of mould present on the agar strips indicates two things. First, that the manufacturer (Biotest Diagnostics Corporation, Denville, NJ) produced quality agar strips free of contamination, and second, that the procedures involved in sampling (cleaning the instrument, handling the agar, sealing the agar strip, and transporting it to the laboratory) did not introduce frequent contaminants.

As indicated in Section 3.3, to obtain proof that the RCS Plus was free of fungal spores and that the cleaning regime was sufficient to remove all mould contaminants, separate trials were conducted in a "clean room". The sample volume for these trials was increased from 300 L to 812 L, resulting in a lower detection limit of 1 CFU/m^3 (Sand, 2001). As illustrated in Table 11, all four trials yielded mould levels that were BDL. Besides indicating that the "clean room" was free of mould at these conditions, the results also showed that the RCS Plus instrument was itself free of mould and was not a source contributing to mould on the agar strips during sampling.

Table 11. Total Fungal Counts Observed During "Clean Room" Testing at 15% Humidity, 22°C and a Volume of 812 L

Trial	Total Fungal Count (CFU/m^3)
1	< 1
2	< 1
3	< 1
4	< 1

Given that neither the sampling technique nor the instrument was a source of mould contamination, the precision of the results is briefly discussed. As described in Section 3.3, duplicate air samples were obtained in most of the schools sampled. Examining raw duplicate results (Appendix 17) revealed that, on a species (or genus) level, the fungal counts obtained for the two consecutive samples differed. (This situation did not apply to instances when both replicates were BDL, which was especially prevalent in the winter sampling round.) For example, duplicate results obtained in the fall sampling round for *Cladosporium* species are illustrated below in Table 12 (these samples were obtained using a 300 L sample volume).

Table 12. Comparing Duplicate Results Obtained for *Cladosporium* Species During the Fall

School Code	*Cladosporium* Count Sample #1 (CFU/m^3)	*Cladosporium* Count Sample #2-duplicate (CFU/m^3)
3	17	10
4	13	13
5	33	17
6	17	40
7	7	10
9	183	57
11	<3	<3
12	<3	<3
13	7	<3
14	13	10
14	3	13
15	<3	10
17	20	10
18	53	20
19	7	<3
20	<3	3
21	17	13
22	<3	23

A nonparametric Wilcoxon matched-pair sign test (Zar, 1974) was completed in order to determine whether the duplicate results for *Cladosporium* species differed in a statistically significant manner (Appendix 18). At a significance level $\alpha = 0.05$,

duplicate results were not deemed as being statistically different. Similar results were also obtained when comparing duplicate results for non-sporulating species (Appendix 18).

Placing these statistical results into perspective however, there were instances when the difference between duplicate values was large. For example, Table 12 School #9, there was a 69% difference between the *Cladosporium* duplicates 183 and 57 CFU/m^3. One reason for these large differences, already stated in Section 3.3, was because the constituents in the air closest to the sampler changed while the samples were being taken. The results obtained from duplicates indicate that it is important to be aware of the variability associated with air sampling, and that the instruments and process of sampling for mould in air is not an exact science.

4.3 Treatment of Below Detection Limit Data

From 202 classrooms and 49 outdoor samples, a total of 24 mould types were identified (some to the species level, others to the genus level). Many indoor samples obtained during the winter sampling round were found to have BDL levels of mould growth. When a particular mould species was not isolated on an agar strip, one of two possibilities existed. Either the mould was not present in the room at time of sampling, or the mould was present in a concentration well below the detection limit of 3 CFU/m^3, which was established with a 300 L sample volume. Table 13 illustrates the percentage of mould samples that were BDL during both sampling rounds.

Table 13. Proportion of Mould Samples Below Detection Limit

Type of Mould	Fall		Winter	
	Indoor BDL (%) n = 141*	Outdoor BDL (%) n = 18	Indoor BDL (%) n = 160	Outdoor BDL (%) n = 31
Acremonium	100	94	99	100
Alternaria	58	**50****	96	100
Aspergillus flavus	100	100	98	97
A. fumigatus	89	**44**	92	71
A. glaucus "group"	84	**22**	94	**16**
A. niger	89	89	98	100
A. versicolor	82	61	90	81
Beauveria	94	94	98	90
Chaetomium	97	100	99	100
Cladosporium	31	28	87	77
Doratomyces	99	100	100	100
Epicoccum	93	100	100	100
Fusarium	99	100	100	100
Mucor	100	100	99	100
Non-sporulating	34	44	87	77
Paeliomyces	98	100	100	100
Penicillium	63	**22**	89	74
Phoma	99	100	100	100
Rhizopus	89	83	98	100
Scopulariopsis	91	**44**	99	77
Trichothecium	99	94	100	100
Ulocladium	98	89	99	100
Verticillium	99	100	100	100
Yeast	100	100	99	100

*replicates are included in calculation of "n" value

**bold values indicate moulds present on 50-90% of agar strips

Bolded values within Table 13 denote moulds that were present on between 50-90% of the agar strips. In fall, the most prevalent moulds indoors were *Cladosporium* and non-sporulating whereas *Alternaria, A. fumigatus, A. glaucus* "group", *Cladosporium,* non-sporulating, *Penicillium,* and *Scopulariopsis* were most abundant outdoors. During winter, no moulds were present indoors on more than 13% of the agar strips. In fact, 92% of the indoor winter data were between 90-100%

BDL. Outdoors during the winter, only one species, *A. glaucus* "group", was present on 84% of the agar strips, whereas 71% of the data were between 90-100% BDL.

As illustrated above, there were 14 instances in the fall, and 22 instances in the winter when a particular mould type was altogether absent on the agar strips (ie. 100% BDL). Of these 14 cases occurring during the fall, 29% were found indoors whereas the majority, 71%, were found outdoors. Of the 22 occurrences of 100% BDLs determined during the winter, 32% were found indoors, whereas 68% were found outdoors. A larger variety of fungi was found indoors (during both seasons) because contaminants were likely concentrated within the building due to lack of adequate ventilation that removed the fungal propagules, whereas outdoors, the wind dispersed the spores to dilute levels. The quantity of 100% BDLs was larger in winter than fall because in the winter, cold temperatures and snow cover decreased the concentration of airborne spores outdoors. When the ground was frozen, mould numbers were lower indoors (compared to fall indoor data) because it was hypothesized that the school occupants were not tracking fungal spores into the building. If however anomalies existed, and significant quantities of mould were detected indoors during the winter, it is less likely that the mould was brought in from the outdoors, but that an amplification and growing site existed indoors. Due to these high proportions of BDL data, statistical analysis was only completed on the most robust set of data, which are highlighted in Table 13. *Cladosporium* species and non-sporulating species were present indoors during the fall 69% and 66% of the time respectively.

Data that are BDL must be dealt with in a systematic, statistically-sound method. Some ad hoc methods include changing the BDL values to the detection limit, others change them to a value of zero, while still others use one half of the method detection limit (Zar, 1974). ACGIH (1989) states that although values of zero are also often dealt with by adding a small constant (eg. 0.1), a better alternative would be to repeat the sample, adjusting the air sampling volume to obtain data within

a measurable range. For this project however, it was not possible to follow this suggestion. As a component of this study to determine if the RCS Plus was free of mould contamination, the "clean room" sampling (Section 4.2) served a second purpose. Recall that the sample volume for these trials was increased from the sampling protocol rate of 300 L to 812 L, thus decreasing the detection limit from 3 CFU/m^3 to 1 CFU/m^3 (Sand, 2001). Sampling 812 L of air during four trials yielded total fungal count results that were below the detection limit of 1 CFU/m^3. Because the results obtained from a much larger sample volume still indicated that no moulds were detected at 1 CFU/m^3, it was assumed that the moulds were not present in the room at time of sampling. Therefore, all results that were BDL were assigned values of 0 CFU/m^3 during statistical analysis. This being said, it should also be noted that making this assumption might have biased the results in a low fashion.

4.4 Descriptive Statistics

Instead of using parametric or nonparametric statistical analysis to draw conclusions, descriptive statistics report general trends in the data. For example, outdoor fungal counts during the fall ranged from 30-800 CFU/m^3 with a median of 180 CFU/m^3, whereas in the winter, they varied from BDL to 530 CFU/m^3 with a median of 46 CFU/m^3. Indoor fungal counts during fall sampling varied from BDL to 650 CFU/m^3 with a median of 33 CFU/m^3, wheras indoor samples in the winter varied from BDL to 160 CFU/m^3 with a median that was also BDL. These results were based on single sample agar strips and were not the averages of classrooms or the sum of duplicates.

4.4.1 Health Canada Guidelines

As indicated in Section 2.5, there are numerous fungal level guidelines that were set up by private and government organizations, and many more recommendations put forth by IAQ investigators. There are several reasons why the results obtained from this study were compared to the 1995 Health Canada guidelines.

Firstly, the WHO (1997) recommends these guidelines. Secondly, the guidelines were derived from a large database of samples which were taken over nine years, lending itself to the credibility of the results. Thirdly, the sampling strategies involved in this study (ie. RCS Plus instrument with RB agar) were similar to those used to obtain the Health Canada guidelines. Fourthly, recommendations put forth by a separate Canadian study which also used an RCS Plus and RB agar (Miller et al., 1988) were similar to those established by Health Canada. Lastly, the Federal-Provincial Committee on Environmental and Occupational Health (1994) state that the Health Canada guidelines were found useful by workers in the field and were being used on a regular basis.

4.4.1.1 Signs of Pathogenic Fungi

Health Canada (1995) identifies *A. fumigatus, E. capsulata* and *Cryptococcus* species to be pathogenic fungi that should not be present in indoor air. Neither *E. capsulata* nor *Cryptococcus* species were observed during either sampling round using the techniques described in Appendix 15. This was perhaps a false negative however, because these two types of fungi are not readily isolated on the broad spectrum RB agar strip, nor by other air sampling techniques (Health Canada, 1995). Health Canada (1995) states however that these moulds are rare in urban environments, and further to this, are not common in Alberta (Sand, 2001).

During the fall sampling round, ten out of 22 schools had concentrations of *A. fumigatus* between 3-40 CFU/m^3, with a median of 13 CFU/m^3. The results for these ten schools are illustrated in Table 14. In all schools except two, the fungus was present in only one of the three classrooms that were sampled. In one school (#8), *A. fumigatus* was detected at the method detection limit (MDL) of 3 CFU/m^3 in two classrooms. In another school (#15), *A. fumigatus* was isolated in all three classrooms, with levels ranging from 13-20 CFU/m^3. Out of the 10 cases, five counts of *A. fumigatus* were equal to or below outdoor counts, two were slightly above outdoor counts, and two could not be compared to outdoor fungal counts because

outdoor samples were not taken due to cold temperatures. The remaining school (#1) that had higher numbers of *A. fumigatus* indoors than outdoors, happened to be resampled during the winter, yielding BDL levels of *A. fumigatus*.

During the winter sampling round, ten out of 31 schools had *A. fumigatus* present in quantities ranging from 3-37 CFU/m^3, with a median of 3 CFU/m^3. The results for these schools are also presented in Table 14. Eighty percent of the time, *A. fumigatus* was present in only one of the five classrooms that were sampled. In one school (#28), *A. fumigatus* was present in three of the five rooms that were sampled, with counts around the MDL of 3 CFU/m^3. Fourty percent of the time, numbers of *A. fumigatus* were lower indoors. From these results, *A. fumigatus* was present at very low numbers in approximately 47% of the schools or 12% of the 202 rooms sampled.

Table 14. Numbers of *Aspergillus Fumigatus* Isolated During Fall and Winter

Fall				Winter			
School Code	Room	Indoor (CFU/m^3)	Outdoor (CFU/m^3)	School Code	Room	Indoor (CFU/m^3)	Outdoor (CFU/m^3)
1	A	23	7	3	B	3	<3
3	C	7	7	4	A	3	10
5	A	7	300	7	A	37	10
8	A	3	<3	25	C	7	10
	B	3		26	A	7	
11	A	17	n/a			3	20
13	A	40		27	A	17	<3
		17	17	28	A	10	
		3			C	3	30
15	A	13			D	3	
	B	13		32	D	3	<3
	C	20	33	35	C	10	<3
		17		42	D	3	<3
19	A	13	23				
20	B	3	n/a				
22	C	7	30				

4.4.1.2 Persistent Presence of Toxigenic Fungi

Continuous presence of toxigenic fungi such as *S. chartarum*, *Penicillium* and *Fusarium* via air sampling could only be determined in the ten schools that were resampled for the seasonality subset. Eight out of the ten schools sampled in fall had between 3-30 CFU/m^3 of *Penicillium* species present in one to three rooms with a median of 10 CFU/m^3. Elevated counts of *Penicillium* species (223 CFU/m^3) were present in one school (#21) and were attributed to the fact that the ventilation system had not been operational for a couple of days prior to sampling due to construction. During winter sampling, *Penicillium* species were present in only four of the ten repeated schools with a range of 3-7 CFU/m^3 and a median of 5 CFU/m^3. In the winter, *Penicillium* species were never present in more than one room per school.

During both fall and winter sampling, *Fusarium* species were not present indoors or outdoors for the ten schools in the seasonality subset. Of the remaining 33 schools that were not part of the seasonality subset, there were only two cases of *Fusarium* species being isolated indoors, both of which were near detection level and during the fall. Persistent presence of *Fusarium* in these two schools would have to be determined through resampling.

S. chartarum was not isolated on any agar strips taken outdoors or in classrooms. This may in part be due to the sticky nature of *S. chartarum* spores that are not easily captured by typical air sampling techniques (Sand, 2001). *S. chartarum* and *S. conidia* were however both found on tape samples taken in the boiler room of one school (#16) where several areas of mould were observed growing on canvas material. A follow-up investigation concluded that the presence of *Stachybotrys* species in the boiler room was not resulting in contamination of classrooms (Probert, 2001).

4.4.1.3 Exceedances of 50 CFU/m^3 Guideline

Concern may exist when more than 50 CFU/m^3 of a single species (excluding tree and leaf fungi *Cladosporium* and *Alternaria*) is found indoors (Health Canada, 1995). During fall sampling, eight instances were found in four schools where either *Penicillium* or *Rhizopus* species exceeded the 50 CFU/m^3 guideline. As previously indicated, in one school (#21), high *Penicillium* values were attributed to a non-functioning ventilation system. *Penicillium* levels ranging from 87-580 CFU/m^3 with a median of 192 CFU/m^3 were present in all three rooms sampled in another school (#10). The outdoor sample for this school yielded no *Penicillium* spores. These high *Penicillium* counts were likely due to the absence of a ventilation system, thereby allowing spores to accumulate in the building, and due to the rural location of this school. It is hypothesized that students living on farms would track in a plethora of fungal spores on their person and suspend the spores in the air through regular room activity, and without a ventilation system, spores would likely accumulate.

A total of three classrooms in two schools (#6 and #22) yielded *Rhizopus* concentrations above 50 CFU/m^3. The elevated numbers of *Rhizopus* species that were found in one room in school #6 during fall were not present during winter sampling, thus was not likely a persistent problem. In school #22, all three rooms that were sampled had between 3-160 CFU/m^3 *Rhizopus* species present, however only two of the rooms exceeded guideline levels. At time of sampling, the outdoor number of *Rhizopus* species was 37 CFU/m^3, thus helping to explain the presence of this species within classrooms.

During the wintertime, three types of mould were isolated in five schools at or exceeding levels of 50 CFU/m^3. In two schools (#30 and #31), *Penicillium* numbers hovered around 150 CFU/m^3 whereas in another two schools (#34 and #39) concentrations of *Penicillium* were both 50 CFU/m^3. In all instances, the outdoor samples for these four schools yielded BDL results for *Penicillium*. Due to elevated numbers of *Penicillium* species, IAQ advisory forms were issued for all four schools

and follow-up investigations were prompted. *Rhizopus* species were also present at 60 CFU/m^3 in a different room within school #34, while the outdoor levels of *Rhizopus* were BDL. Finally, 80 CFU/m^3 of *A. versicolor* were isolated in school #32, and similarly, the outdoor sample did not detect any *A. versicolor* spores. These high indoor levels were likely due to inefficient ventilation systems that are concentrating mould within the rooms.

In summary for both seasons, 21% of schools or 7% of rooms sampled contained levels of *A. versicolor, Pencillium,* or *Rhizopus* species that were equal to or that exceeded Health Canada's (1995) guideline of 50 CFU/m^3. A slightly higher proportion of these exceedances occurred in schools sampled during fall.

4.4.1.4 A Mixture of Species in Exceedence of 150 CFU/m^3

Health Canada (1995) states that a total fungal level of up to 150 CFU/m^3 is acceptable if there is a mixture of species that are reflective of the outdoor sample. Although most schools meet this requirement, several schools did not. In the fall, four samples in three schools had fungal numbers that were greater than 150 CFU/m^3 for a mixture of species. For example, one room in a school (#2) had a total count of 150 CFU/m^3, consisting of 82% *Alternaria*, 8% *Cladosporium*, 5% *Penicillium*, and 5% non-sporulating species. The total count for the outdoor sample was 59 CFU/m^3, including 20 CFU/m^3 *Cladosporium* (34%), 3 CFU/m^3 *Penicillium* (5%), 13 CFU/m^3 non-sporulating (22%), and BDL *Alternaria* (0%). Comparing indoor and outdoor signatures, all but the *Alternaria* species appeared indoors in less than or equal to outdoor concentrations.

One replicate sample in a classroom of the second school (#9) yielded a total CFU/m^3 of 210. Indoor components included 87% *Cladosporium*, 10% non-sporulating, and 3% *A. versicolor* species. The total count for the outdoor sample was 187 CFU/m^3, and consisted of 67 CFU/m^3 *Cladosporium* (36%) and non-detectable

levels of non-sporulating and *A. versicolor* species. In all cases, higher levels of *Cladosporium*, non-sporulating, and *A. versicolor* species were found indoors.

Determining if outdoor species were reflective of indoor species found in two classrooms in the third school (#22) was demonstrated best in Table 15. One air sample was taken in each room, resulting in a total fungal count of 217 CFU/m^3 in room A and 196 CFU/m^3 in room B.

Table 15. Comparing Indoor and Outdoor Fungal Types Found at School #22

Outdoor (186 CFU/m^3 total)	Indoor Room A (217 CFU/m^3 total)	Indoor Room B (196 CFU/m^3 total)
19% *Rhizopus*	74% *Rhizopus* (+)	29% *Rhizopus* (+)
7% *Penicillium*	18% *Penicillium* (+)	30% *Penicillium* (+)
11% *A. versicolor*	17% *A. versicolor* (+)	5% *A. versicolor* (-)
18% *Cladosporium*		22% *Cladosporium* (~)
12% non-sporulating		7% non-sporulating (-)
6% *Alternaria*		5% *Alternaria* (~)
11% *A. niger*		2% *A. niger* (-)

Legend: (+) greater than outdoor
(-) less than outdoor
(~) approximately equal to outdoor

As indicated by symbols in Table 15 that denote whether a particular fungal type indoors was larger than, less than, or approximately equal to its outdoor equivalent, *Rhizopus* and *Penicillium* species were present in higher quantities indoors, indicating a concentration of those spores in some parts of school #22. Repeated air-sampling in this school would be required to determine if *Rhizopus* and *Pencillium* levels persistently exceeded guideline levels.

During winter sampling there were no air samples that exceeded 150 CFU/m^3 in a mixture of species. To summarize these findings, only 2% of rooms or 7% of schools sampled exceeded the Health Canada guideline of having a mixture of species exceeding 150 CFU/m^3.

4.4.1.5 Tree or Leaf Fungi Less than 500 CFU/m^3

Up to 500 CFU/m^3 is deemed an acceptable fungal number in the summer time only if the species are predominantly *Cladosporium* and *Alternaria* or other tree and leaf fungi (Health Canada, 1995). Because this study was not completed during the summer months, there were no instances where tree or leaf fungi should be permitted to reach levels of 500 CFU/m^3. Although not cause for concern, there were two instances, both occurring in the fall, where *Alternaria* or *Cladosporium* exceeded 100 CFU/m^3. In one school (#2), *Alternaria* reached concentrations of 123 CFU/m^3, whereas in another school (#9) *Cladosporium* was detected at 183 CFU/m^3.

4.4.1.6 Visible Presence of Fungi

Health Canada (1995) states that any presence of visible fungi should be remediated, regardless of airborne spore load. Besides the boiler room in which *S. chartarum* was isolated in one school (#16), there were no instances where visible fungi were present in classrooms.

4.4.2 Comparing Results to Literature School Values

Fungal air sampling conducted in schools in other studies were compared to results of this study. From the sampling of 631 schools in Atlantic Canada, Rand (1998) determined that non-contaminated schools have low proportions of soil dwelling species (*Aspergillus* and *Penicillium*) to phylloplane species (*Alternaria, Cladosporium, Ulocladium* and yeasts). During the fall sampling round of the present study, fungal numbers in seven of 70 rooms were determined to have greater than ten times the amount of soil-dwelling species to phylloplane species. These values were found in four schools, #10, #15, #21, and #22, all of which have been previously mentioned as having atypical fungal levels. For example, schools #10, #21 and #22 all had elevated numbers of *Penicillium* species, whereas school #15 had no phylloplane species in two of the rooms, and had a combination of *A. fumigatus, A. glaucus* "group", *A. versicolor* and *Penicillium*.

During winter, fungal numbers in seven of 132 rooms were determined to have at least ten times the amount of soil dwelling species to phylloplane species. These values were found in seven different schools (#7, #27, #30, #31, #32, #39 and #40), three of which (#30, #31, #39) have been identified as having elevated concentrations of *Penicillium* species. Four classrooms in the remaining schools had combined *Aspergillus* species numbers ranging from 37-80 CFU/m^3, and did not have any phylloplane species.

Table 5. (Section 2.4.2) summarized previous fungal sampling research conducted in schools. Within these eight studies, total fungal counts ranged from BDL-6450 CFU/m^3 with the most common genera being *Cladosporium, Penicillium*, and *Aspergillus*. Previous studies conducted in Canada indicated that total fungal numbers ranged from non-detectable to 350 CFU/m^3. In this study, fungal counts ranged from BDL-650 CFU/m^3, and the most prevalent mould types found indoors during the fall were *Cladosporium*, and non-sporulating.

4.5 Nonparametric Statistics

As indicated in Section 2.9, nonparametric statistical analysis is often used when dealing with bioaerosol data such as mould counts. Because there were so many samples with BDL counts during the winter sampling round, limited statistical analysis were completed on this data set. As was previously mentioned, statistical analysis was only completed on the most robust set of data (*Cladosporium* and non-sporulating species) which were present indoors during the fall 69% and 66% of the time respectively.

4.5.1 Most Prevalent Fungal Types Found Indoors and Outdoors

A total of 21 fungal types were detected indoors during fall, while in winter, 16 types were found. From Table 13 (Section 4.3), the two most prevalent types occurring indoors and outdoors during fall were determined to be *Cladosporium* and

non-sporulating species. There were no prevalent mould types found indoors during winter.

Histograms shown in Figure 8 for indoor and outdoor levels of *Cladosporium* and non-sporulating species during fall illustrate the skewed (non-normal) nature of the data. Because many samples were below the detection limit, nonparametric tests were used.

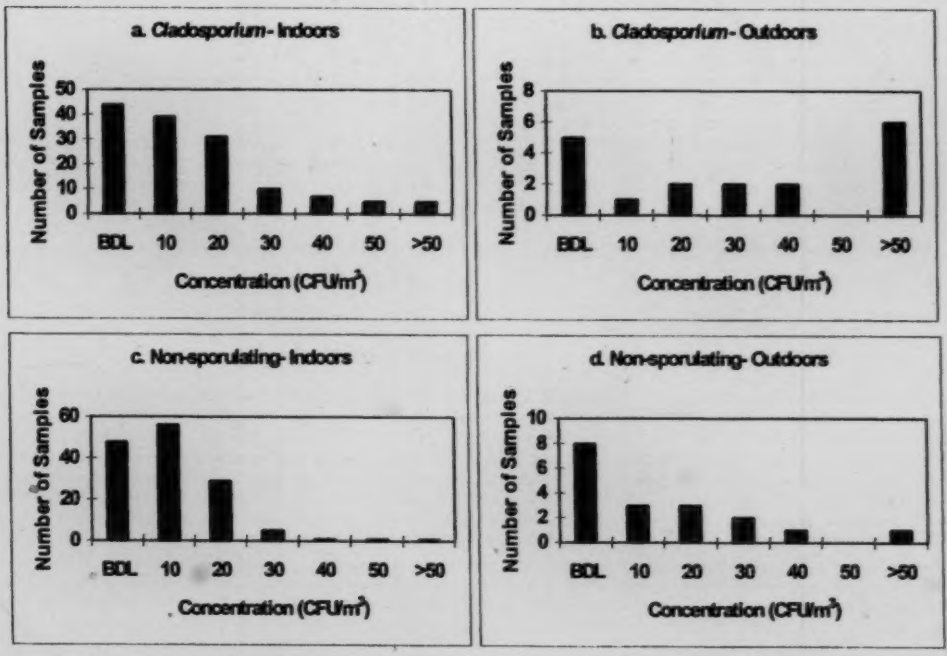
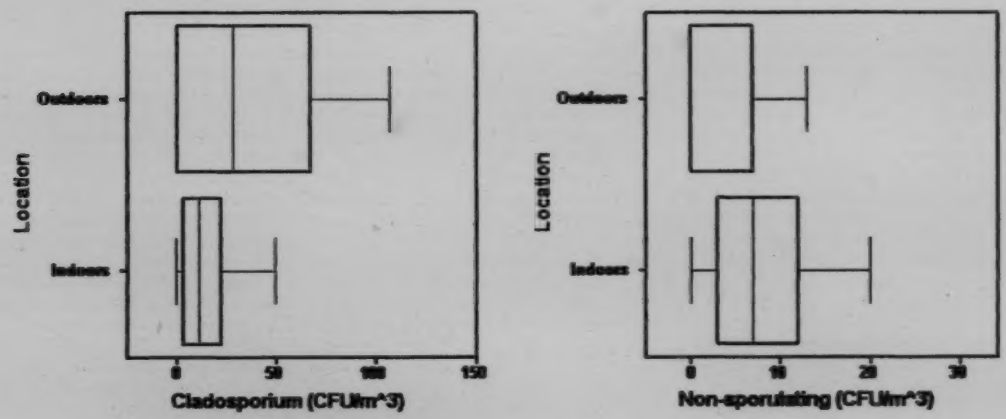


Figure 8. Histograms of *Cladosporium* and Non-sporulating Species both Indoors and Outdoors During the Fall.

4.5.2 Comparing Indoor and Outdoor Fungal Concentrations

Using the most robust data sets (obtained from *Cladosporium* and non-sporulating species), a comparison of indoor and outdoor numbers during the fall was completed using Wilcoxon's nonparametric matched-pair sign test (Appendix 19). At

a significance level α=0.05, the concentrations of *Cladosporium* species were found to be lower indoors than outdoors, whereas levels of non-sporulating species indoors and outdoors were not distinguishable. Figure 9 illustrates *Cladosporium* and non-sporulating data through use of box-and-whisker plots. The box represents the interquartile range (IQR) which contains 50% of the data, between the 25th and the 75th quartile. The whiskers extend from the box to the highest and lowest values, excluding outliers, and the line across the box indicates the median. An outlier is an unusually large or small value in a data set, which is far removed from the 25th or the 75th quartile (Khazanie, 1990).



Legend: **Box** - interquartile range (IQR)
Whiskers - highest and lowest values
Line across box - median

Figure 9. Variability Observed Between Indoor and Outdoor Samples for *Cladosporium* and Non-sporulating Species

From Table 16 it can be seen that there were no discernible differences between numbers of non-sporulating species indoors and outdoors, because their median, IQR and maximum values were quite similar. There were however discernable differences in *Cladosporium* numbers between the two locations because of a large difference in maximum values and IQRs.

Table 16. Descriptive Statistics for *Cladosporium* and Non-sporulating Species During the Fall, both Indoors and Outdoors

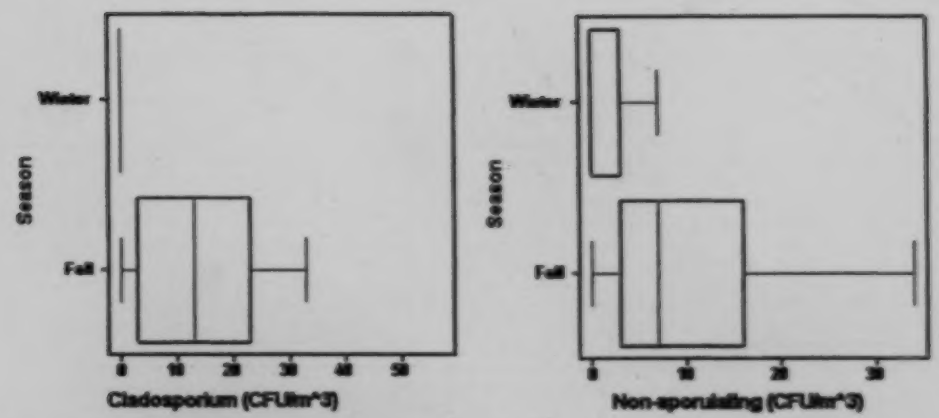
	Indoor Samples (n = 141) (CFU/m^3)			Outdoor Samples (n = 18) (CFU/m^3)		
Fungus	**Max**	**Median**	**IQR**	**Max**	**Median**	**IQR**
Cladosporium	183	13	16	617	33	80
Non-sporulating	67	10	13	73	17	20

4.5.3 Subsets

Recall that subset sampling was conducted in order to determine sampling bias. Jhangri (2001) stated that these tests must be completed on a species level and for the most prevalent species with the most robust data set, hence they were only completed for *Cladosporium* and non-sporulating species. Because statistical analysis was completed using data from only *Cladosporium* and non-sporulating species, the results below are likely only valid for common types of moulds originating from outside air, and not for those that are reproducing indoors and that are tightly adhered to indoor surfaces.

4.5.3.1 Seasonal Variability

In order to test the hypothesis that fungal numbers of the predominant species were the same concentration in fall and winter, a Wilcoxon matched-paired sign test was performed (Zar, 1974). This test is a nonparametric equivalent to the paired-sample t-test, however no assumptions were made about whether the data are from a normal distribution. These data were evaluated for both *Cladosporium* and non-sporulating species, which were the most abundant species during fall. In both cases, the null hypothesis was that there would be no difference observed between mould numbers in fall and winter. At a significance level α=0.05 the null hypothesis was rejected, and a difference between fall and winter *Cladosporium* levels and non-sporulating species levels was concluded (Appendix 20). Via a box-and-whisker plot, Figure 10 summarizes the descriptive statistics for *Cladosporium* and non-sporulating species over both seasons.



Legend: **Box** - interquartile range (IQR)
Whiskers - highest and lowest values
Line across box - median

Figure 10. Seasonal Differences in *Cladosporium* and Non-sporulating Species Levels

Although other species observed within this study were not subjected to this nonparametric test, in general, winter fungal numbers were lower than fall numbers (Appendix 17). The results of this study are comparable to those found in the literature (Larsen, 1981).

4.5.3.2 Within Space Variability

Nine schools were randomly selected to help determine the variability of fungal levels occurring within space. The RCS Plus was set up at three different locations within the three rooms sampled per school. Figure 11 depicts a typical example of the variability of fungal counts observed within a particular room. As illustrated below, *A. glaucus* "group" species were present in all three sampling locations, ranging from 6-17 CFU/m^3. *Alternaria*, *Cladosporium*, and *A. fumigatus* species however, were present in only two of the three sampling locations, whereas *Penicillium* species were only present in one of the locations.

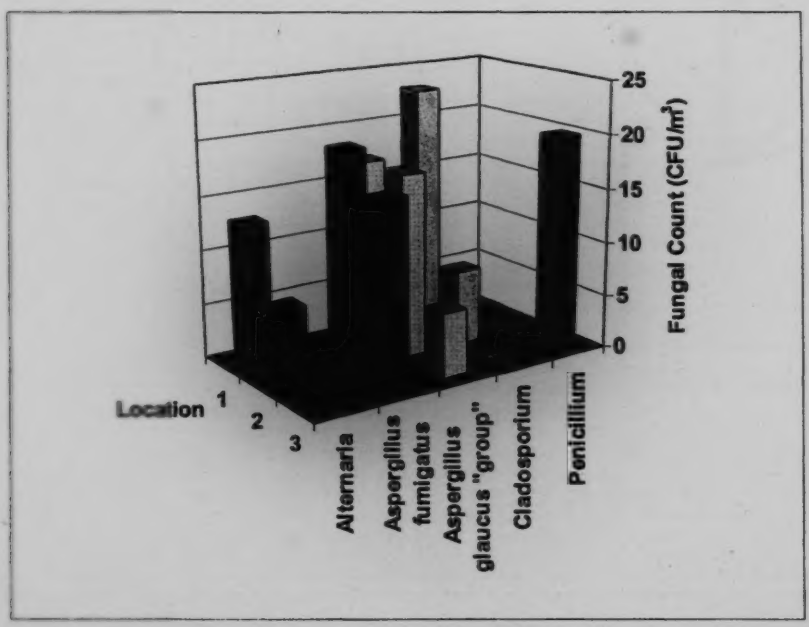


Figure 11. Typical Fungal Count Variability Observed when Sampling in Three Locations within the Same Room

Descriptively, there were differences in fungal levels within the same room. In order to determine whether the concentration of mould levels in each location was statistically significant however, a large number of replicates would have been required per room. This would have been disruptive to occupied classrooms as the instrument made a slight whirring noise, and investigators would have had to place the instrument right in the middle of students in order to adequately represent the whole room. IAQ investigators must weigh the cost and time required in taking numerous measurements in one space against being able to accurately depict a room's mould characteristics in order to report the findings in a confident manner. Other results obtained from the nine schools in which variability within space was investigated are provided in Appendix 21.

4.5.3.3 Within Phase Variability

Originally, variability within phases was going to be determined using winter sampling data. Because there were too many BDL values, these data were not amenable to statistical analysis. Because all schools and rooms were randomly selected, it was decided that the most robust set of data from fall sampling would be used to determine whether there was a within phase effect on fungal counts of *Cladosporium* and non-sporulating species. Matched pair samples were used to compare two rooms within the same phase within each school resulting in a total of 19 matched pairs. At a significance level α=0.05, the Wilcoxon paired-sample test (Zar, 1974) revealed that, for both *Cladosporium* and non-sporulating species, room A was not found to be different in fungal levels than room B within the same phase (Appendix 22). Figure 12 illustrates similarities between medians and IQRs obtained in room A and B for the two most prevalent species.

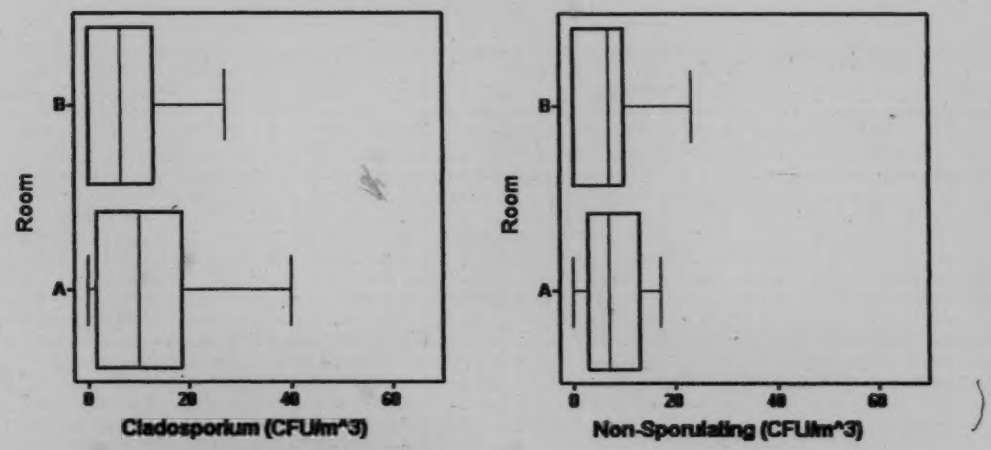


Figure 12. Variability Within Phase for the Two Most Common Species During Fall

Similarities observed between room A and B within the same phase may be due to similar ventilation systems servicing both rooms (ie. both unit ventilators, or from the same central system). It may also be due the imprecision of the RCS Plus that minor fungal concentration differences were not detected between the two rooms within the same phase. In future fungal studies within CHA region schools, investigators will be able to obtain a random sample of rooms within the same phase without concern of introducing bias into the results.

4.5.3.4 Between Phase Variability

Because there were too many BDL data collected during winter, variability between phases was statisically analyzed using 11 schools that met criteria from the fall sampling round. Of the three classrooms that were sampled within each school, at least two of them must have been from different phases in order to compare differences between room A in phase I and room B in phase II. If all three classrooms met this criterion, then two of them were randomly selected to complete a Wilcoxon matched-pair sign test (Zar, 1974). Again, the most robust species, *Cladosporium* and non-sporulating, were utilized to complete statistical analysis. The null hypothesis was that there were no differences between fungal concentrations within room A in phase I and room B in phase II for *Cladosporium* or non-sporulating species. The null hypothesis was rejected for *Cladosporium* species at a significance level of $\alpha = 0.05$, indicating that the fungal results in rooms of two different phases were not similar. At the same level of significance ($\alpha = 0.05$), the non-sporulating species concentration was determined to be similar in both phases. In future studies, due to the conflicting statistical results, fungal contaminants should not be assumed to be similar in rooms within different phases (Appendix 23). This may in part be due to different ventilation systems and to different building materials. Figure 13 illustrates the medians and IQRs obtained within room A in phase I and room B in phase II for the two most prevalent species.

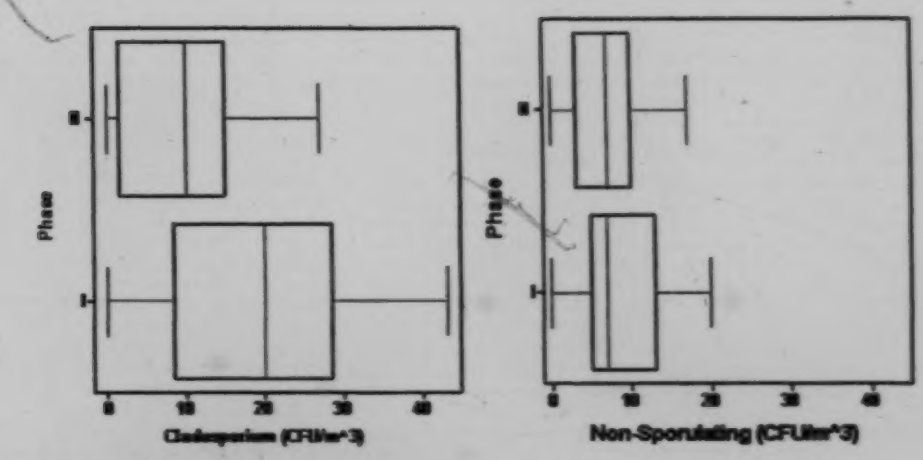


Figure 13. Variability Between Phases for *Cladosporium* and Non-sporulating Species During Fall

4.5.3.5 Temporal Variability and Occupant Activity

School #10 did not have any source of ventilation and only used convection heat. As indicated in Section 4.4.1.3, *Penicillium* concentrations from samples in this school were above Health Canada's (1995) guidelines, indicating a source of amplification. A follow-up investigation was triggered, and a CHA representative resampled some of the rooms containing high *Pencillium* numbers (Probert, 2001). The methodologies were not precisely the same as the sampling protocol in the present study, however air samples were taken with a RCS Plus instrument on RB agar strips. One difference in the experimental procedure of the follow-up investigation was that it was conducted in completely unoccupied rooms. Fungal numbers obtained from this follow-up investigation indicated that *Penicillium* numbers were well below the 50 CFU/m^3 guideline. It was speculated that in the absence of students resuspending dust and spores, the spores had settled and were not

in the air. Another possibility was that many students in the area were from the farm and were carrying mould-containing hay, grain, and dust into the school.

4.6 Experimental Error

Errors are inherent to any experiment. Four main experimental errors were identified within this study: freezing agar, sampler placement, cross-contamination of samples, and handling of agar strips.

4.6.1 Sub-zero Sampling Temperatures

Because the RB agar crystallized outside when temperatures were below 0°C, it is likely that the results obtained for the outdoor samples during the winter are biased low. Although spores still impacted onto the agar strip, it was possible that some of the frozen spores did not grow into colony forming units during incubation. Based on information provided by the RB agar manufacturer (Biotest, 2001), the agar should maintain growth of fungi after being briefly frozen, but it may be more difficult to enumerate if there is too much liquid inside the strips. Mycology laboratory staff never encountered liquefied agar, likely because the samples were only frozen on the top layer as the sample volume was reduced during cold temperatures (Sand, 2001).

4.6.2 Sampler Placement

As previously indicated, due to the configuration of classrooms, the RCS Plus was usually not located in the center of the room. In some very congested classrooms, the instrument was placed near walls in order to minimize disruption of the class, and because there was no other place to put it. When the instrument was placed near walls, it was in a location of less air mixing, which would not be completely representative of the whole room, as might be the center of the room (ACGIH, 1989). And sometimes the RCS Plus was not placed on its tripod due to limited space, and therefore was not always at the breathing height of students.

4.6.3 Cross-contamination of Samples

It is possible that, even with cleaning, cross-contamination from previously high fungal count samples occurred. This would be considered a random error, and could not be predicted at time of sampling. Between agar samples, this error was minimized by cleaning between samples and by investigators donning new gloves. Between school sampling, soaking the impeller in a 70/30 isopropyl/water mixture over night minimized cross-contamination from one school to another. If the impeller was not completely dry before the first use, it is possible that the results would be biased low as the alcohol would serve to disinfect the first agar sample. A wet impeller was rarely observed, and would not be considered to be adding a large source of error to the data.

4.6.4 Handling of Agar Strips

Literature states that exposure of RB agar to light photoactivates a fungicide (Boss and Day, 2001). Every morning the agar strips were transferred from a Styrofoam container in a fridge to a plastic bag in an opaque cooler. The only time the agar was exposed to light was when it was being prepared for sampling. In some instances however, it was noted that during sampling, the cooler lid was not always tightly fitted, thus allowing some light to penetrate. This error would serve to decrease fungal levels observed on the agar strips.

5.0 CONCLUSIONS AND RECOMMENDATIONS

One of the main research objectives of this study was to determine baseline concentrations of mould across all schools within the Capital Health Authority district. These baseline data will help future IAQ investigators to determine whether fungal problems exist in schools with IAQ complaints. While meeting this major goal, several smaller goals were achieved. For example, five survey tools were constructed in order to capture school conditions, which provide background information to the samples that were taken. Also, a sampling protocol for identifying fungal contaminants in schools was also established and can be used for future reference.

- Overall, significant fungal problems within Edmonton Public, Elk Island Catholic, and Elk Island Public schools were not observed, however there were some instances where the Health Canada (1995) guidelines were exceeded. Fourteen percent of schools were issued IAQ advisories due to *Penicillium* numbers exceeding Health Canada's (1995) guideline. Upon further investigation of these rooms however, *Penicillium* numbers were well below the guideline. It was recommended that one school (#22) be resampled in order to determine if there is a persistent presence of elevated numbers of *Rhizopus* species, which were present in all three rooms that were sampled.

- The pathogenic species *Aspergillus fumigatus* was observed in 12% of the 202 rooms sampled in quantities varying from 3-20 CFU/m^3, which do not constitute elevated levels. Four schools were observed to have repeated presence of *Penicillium* species during the fall and winter sampling rounds, but in such low numbers (ie. 3-7 CFU/m^3) that concern was not warranted. Subsequent investigation of the visible presence of *Stachybotrys* species within the boiler room of school #16 concluded that contamination had not occurred in classrooms (Probert, 2001).

- During the fall, 15 of 24 of fungal types detected were ≥90% BDL whereas during winter, 92% of all fungal types detected were ≥90% BDL. Due to the large proportions of data that were BDL, many mould types were excluded from nonparametric statistical analysis. *Cladosporium* and non-sporulating species were found to be the most prevalent species indoors during fall, and were thus used to complete statistical analysis. A difference between indoor and outdoor concentrations of *Cladosporium* was found, whereas there was no observed difference for non-sporulating species.

- The results from the nonparametric statistical analysis, which only used data from *Cladosporium* and non-sporulating species, are likely only valid for common moulds originating from outside air, and not for those that are reproducing indoors and that are tightly adhered to indoor surfaces. Analysis of subsets led to several conclusions. First, at a significance level of $\alpha = 0.05$, a statistically significant difference between fall and winter fungal numbers was observed for both *Cladosporium* and non-sporulating species. Second, descriptively, there was some variability of fungal presence within the same space, though not necessarily at a statistically significant level. Third, nonparametric analysis concluded that there were no differences in *Cladosporium* and non-sporulating species observed when sampling two rooms within the same phase ($\alpha = 0.05$). Fourth, the variability of fungal numbers occurring between phases was not clear. At a significance level of $\alpha = 0.05$, results from a Wilcoxon matched-paired analysis concluded that there was a statistically significant difference between the *Cladosporium* numbers within rooms in two different phases. Using the same nonparametric analysis for non-sporulating species however, concluded that there were no differences between non-sporulating numbers in two rooms within different phases. And last, temporal variation existed in which resampling the same location at different times produced different fungal results.

- Several concepts should be kept in mind for future work while investigating school complaints within the CHA region. As there were some differences observed

in fungal numbers within the same room, it is recommended that replicates be taken within each room in order to give a more complete picture of a room's fungal contaminants. For future reference, it is important that the sampling protocol be adjusted to reflect various geometric shapes of the rooms, and that the RCS Plus must be set up where class disruption is minimal (which might not necessarily be in the center of the room). Furthermore, to minimize non-detectable values, the sample volume of the RCS Plus should be increased to at least 812 L, especially when sampling indoors during the winter. And lastly, rooms should always be sampled when they are occupied in order to represent regular room activity.

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7.0 APPENDICIES

Appendix 1. Microbiological Air Samplers

Air Sampler	Description	Cutoff Point*
1. Slit-to-Agar Impactor		
Mattson-Garvin Air Sampler	• 28 L/min air drawn through a 0.15 x 41-mm slit and impacted onto a semisolid medium on a rotating turntable	d_{50}: 0.5 μm
New Brunswick Slit-to-Agar Air Sampler	• 15-55 L/min air drawn through 25 mm long x 0.1 to 0.2-mm wide slit and impacted onto agar on a rotating turntable	d_{50}: unknown
2. Multiple-Hole		
Burkard Portable Air Sampler for Agar Plates	• 10 or 20 L/min of air drawn through 100 1 mm holes onto agar	d_{50}: 4.18 μm at 10 L/min d_{50}: 2.56 μm at 20 L/min
Andersen Single Stage Viable Particle Sampler	• 28 L/min of air drawn through 400 0.25-mm holes onto agar • Works by pulling air through multiple jets below which is placed a Petri dish containing appropriate agar medium (AIHA, 1996)	d_{50}: 0.57 - 0.65 μm
Surface-Air-Samplers	• 180 L/min of air drawn through 220 holes onto agar	d_{50}: 1.35 - 1.9 μm
Andersen Two Stage Viable Sampler	• 28 L/min of air drawn through 2 stages with 200 1.5 or 0.4-mm holes onto agar	d_{50}: Stage 0 - 6.28 μm d_{50}: Stage 1 - 0.83 μm
Andersen Six Stage Viable Sampler	• 28 L/min of air drawn through 6 stages with 400 holes onto medium • Hole diameters stages 1-6: 1.18, 0.91, 0.71, 0.53, 0.34, 0.25-mm • Smaller particles follow the gas streamline, and the larger ones with inertia will deviate and impact onto one of the stages.	d_{50}: Stage 1 - 7.0 μm d_{50}: Stage 2 - 4.7 μm d_{50}: Stage 3 - 3.3 μm d_{50}: Stage 4 - 2.1 μm d_{50}: Stage 5 - 1.1 μm d_{50}: Stage 6 - 0.65 μm

Air Sampler	Description	Cutoff Point*
3. Centifugal Samplers		
Reuter Centrifugal Sampler (RCS)	• Draws 40 L/min of air onto isolation medium contained in plastic strips with 34 wells ~ 1 cm^2 each (Mehta et al., 1996)	d_{50}: ~5 μm
RCS Plus	• Draws 50 L/min • Air flow can be calibrated using a digital flywheel anemometer (Mehta et al. 1996).	d_{50}: ~ 2 μm
RCS High Flow Microbial Air Sampler	• Draws 100 L/min	d_{50}: unknown
AEA Technology PLC Aerojet Cyclone	• Wetted cyclone sampler • Air drawn at 167 or 500 L/min through either a 1.24 or 2.15-cm orifice connected to the body of glass cyclone by elliptical pipe work, and then impacted onto a thin liquid layer (AIHA, 1996)	d_{50}: 0.8 μm at 167 L/min d_{50}: 1.5 μm at 500 L/min
MicroBio MB3 Portable Cyclone Sampler for Bioaerosols	• Wetted cyclone sampler • 167 L/min of air drawn through 1.0-cm orifice	d_{50}: 0.8 μm
Burkard Cyclone Sampler	• Dry cyclone sampler • 10 L/min of air drawn through 0.9 x 0.3-cm vertical orifice into a reverse-flow cyclone	d_{50}: 1.2 μm
4. Filters		
Gin filters	• 1-5 L/min of air • 25, 37 or 45 mm filters in disposable cassettes (1, 2, 5 μm) • Used for heavily contaminated sites (Macher et al., 1995).	

Air Sampler	Description	Cutoff Point*
5. Liquid Impingers		
Burkard Multiple Stage Liquid Impinger	• 20 L/min of air drawn through 3 stages and impacted against wetted surface • Air is drawn through a curved inlet and subsequently through the jet, and then impinged into the liquid (AIHA, 1996) • Offers the advantage that high concentration samples can be diluted with sterile liquid, and low concentration samples can be concentrated by centrifugation (Macher et al., 1995).	d_{50}: Stage 1 - >10 μm d_{50}: Stage 2 - 4 to 10 μm d_{50}: Stage 3 - <4 μm
All Glass Impingers AGI-4, AGI-30	• 12.5 L/min through 1-mm capillary get and impacted on wet surface	d_{50}: 0.3 μm

Adapted from ACGIH (2001)

*An impactor's cut-off point of d_{50} is the particle aerodynamic diameter for which 50% of the particles are collected (Macher et al., 1995).

Appendix 2. Additional References

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Appendix 3. Health Research Ethics Board Form

SECTION A: GENERAL INFORMATION

A1. Project Title
Title of Project: *Indoor Air Quality in Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools*

A2. Applicant Information			
Name: Lorelei Betke, Corinne Stocco, Mary Unobe Title: Graduate Students Department: Environmental Engineering			
Mailing Address: #304, Environmental Engineering Building, U of Alberta			
City & Province: Edmonton, Alberta	Postal Code: T6G 2G7	Phone: 492-8548	Fax: 492-8289
E-mail Address: lbetke@ualberta.ca, cstocco@ualberta.ca, munobe@ualberta.ca			
Signatures: _____ _____ _____			Date:

A3. Co-Applicant Information			
Name: Dr. Warren Kindzierski Title: Associate Professor Department: Environmental Engineering			
Mailing Address: #304, Environmental Engineering Building, U of Alberta			
City & Province: Edmonton, Alberta	Postal Code: T6G 2G7	Phone: 492-0247	Fax: 492-8289
E-mail Address: warren.kindzierski@ualberta.ca			
Signature:			Date:

A3. Co-Applicant Information			
Name: Steven Probert Title: Air Quality Specialist Department: Community Care and Public Health, Capital Health Authority			
Mailing Address: Suite 300, 10216-124 Street, Edmonton, Alberta, T5N 4A3			
City & Province: Edmonton, Alberta	Postal Code: T5N 4A3	Phone: 413-7933	Fax: 482-5383
E-mail Address: sprobert@cha.ab.ca			
Signature:			Date:

A4. Authorizing Signature	
Indication of Department Support for the Implementation of the Project.	
Name of Dept. Chair, Assoc. Dean of Research, or Supervisor: Dr. Terry Hrudey	
Title: Professor and Chair of Civil and Environmental Engineering	
Signature:	Date:

A5. Co-Investigators / Thesis Committee		
Is this project for a graduate thesis? (√) Yes ( ) No *If yes, please provide the names, departments, and phone numbers of your thesis committee.*		
Name:	*Department/Program:*	*Phone:*
Warren Kindzierski	Environmental Engineering	492-0247
Selma Guigard	Environmental Engineering	492-8585
Jerry Leonard	Agricultural Food and Nutritional Science	492-0107

A6. Expedited Review	
*If the study procedures are **LIMITED** to any of the following, please check (√):*	
	Analysis of blood, urine, or any other biological specimen already collected.
	Examination of patient, medical, or institutional records.
	Modification of a previously approved protocol
	Secondary analysis of data.
	Use of biological specimens normally discarded.

A7. Type of Investigation			
Which one of the following best describes the type of investigation proposed? Check (√) more than one if appropriate.			
	Clinical Trial		Multi-centre Trial
	Drug Study		Pilot Study
	Epidemiological Study		Qualitative Study
	First Application in Humans		Technology Assessment / Development
	Sequel to Previously Approved Project (specify title and approval date):		
√	Other (specify): Indoor air quality study.		

A8. Site of Research	
Where will the research be conducted? Check (√) more than one if appropriate. Specify the area/department/program.	
Alberta Cancer Board Sites:	
	Cross Cancer Institute:
Capital Health Authority Sites:	
	Community Care and Public Health:
	Glenrose Rehabilitation Hospital:
	North Edmonton Community Health Centre:
	Royal Alexandra Hospital:
	Stollery Children's Health Centre:
	Sturgeon Community Hospital and Health Centre:
	University of Alberta Hospital:
Caritas Health Group Sites:	
	Edmonton General Hospital:
	Grey Nuns Community Hospital and Health Centre:
	Misericordia Community Hospital and Health Centre:
University of Alberta Sites:	
	Specify (e.g. Corbett Clinic):
Other:	
√	Specify: Schools within Capital Health Region and under the jurisdiction of Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools.
Letters of Support:	
( ) Pending (√) Attached ( ) Not Applicable	

A9. Funding / Budget	
How is the project funded? Please check (√) the appropriate box.	
√	Funding approved; specify source(s): Edmonton Community Lottery Board and Strathcona Community Lottery Board
	Funding pending; specify source(s):
	No external funding required.
Budget	
√	Please check here (√) that you have attached a budget summary. The summary must include details of investigator payments and recruitment incentives (if present). Please attach the budget as an appendix to the form.

A10. Remuneration	
Are any of the investigators involved receiving any directs personal remuneration or other personal or family financial benefits (either direct or indirect) for taking part in this investigation?	
√	Yes. If so, append a letter detailing these activities. Please attach this letter to your budget summary.
	No.

A11. Safety Approvals					
Please check (√) whether or not this study requires any of the following safety approvals. If a safety approval is needed, please indicate whether the approval documentation is pending or attached as an appendix to this form.					
Biohazardous Materials:					
√	Not Applicable		Pending		Attached
Electromechanical:					
√	Not Applicable		Pending		Attached
Health Protection Branch or Other Canadian Federal Agency:					
√	Not Applicable		Pending		Attached
Radiation:					
√	Not Applicable		Pending		Attached

SECTION B: DETAILS OF PROJECT

Description of the Project

B1. Provide a clear statement of the purpose and objectives of the project.

Indoor environmental quality (IEQ) refers to physical, chemical and biological characteristics of the indoor environment (Project Proposal, 2000) and includes parameters such as lighting levels, noise, health and safety as well as indoor air quality (IAQ). Capital Health Authority, Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools are in partnership with the University of Alberta to investigate IAQ in schools in the Capital Health Region. The **purpose** of this two-year study is to determine the status of mould in schools so that responsible authorities may develop an IAQ management plan. The specific IAQ parameters this study investigates are fungi, carbon dioxide, carbon monoxide, temperature, relative humidity, and ventilation rates.

The investigation of indoor air quality in schools within the Capital Health Region arose for several reasons. First, literature states that school IAQ affects student health. Second, Health Canada's Environment Health Directorate recommended that Canadian research initiatives be implemented to establish baseline levels of fungal contamination within public buildings (Env. Health Directorate, 1995). Third, some schools in the Capital Region have had IAQ problems, including the presence of mould. And fourth, there is public pressure with respect to school IAQ, fueled by parental and media attention (Project Proposal, 2000).

Many studies have already investigated IAQ in schools across North America and Europe, and this needs to be investigated in the Edmonton area. Since it is impossible to generalize the results of IAQ studies over geoclimatic regions, a study in the mostly cool and dry Edmonton area, supporting a different fungi population, is required. There are four main research **objectives** in this study to determine presence of fungal contaminants in schools:

- Develop local procedures and protocols for identifying, evaluating, and managing fungal contaminants in schools.
- Identify conditions that contribute to fungal growth in schools.
- Determine baseline levels and types of school fungal contaminants as well as associated indoor air quality parameters.
- Develop cost effective fungal prevention and remediation strategies.

B2. State the hypotheses and/or research questions.

- What is the state of IAQ within schools in the Capital Health Region?
- What are the types and concentrations of fungi in the Capital Health Region schools?
- What conditions facilitate fungal growth?

B3. Briefly summarize past human and/or animal research that has lead to this project.

Background

There are approximately 1,000,000 known species of fungi, and 2,000 new species are identified each year (Miller, 1992). Fungi's quick and asexual reproduction cycle results in the formation of an enormous quantity of spores (Gravesen, 1979). Fungi are heterotrophic and their food demands range over a broad spectrum of organic materials (Gravesen, 1979). In the context of this research, fungi are mainly mesophilic with an optimal temperature for growth between 20-40°C, and prefer more acidic conditions around pH 5-6 (Gravesen, 1979). Most spores in indoor air originate from the outdoors, (Pasanen, 1992) and readily enter by circulating through doorways, windows, and heating, ventilation and air-conditioning (HVAC) systems (American Academy of Pediatrics, 1998). Fungus can grow on virtually any substrate including: glass, paint, rubber, textiles, electrical equipment, and organic material such as paper, cardboard, ceiling tiles and wood products (Miller, 1992). There are three main factors that contribute to their growth: sufficient water, mild temperatures, and organic nutrient.

Adverse health effects

Between 6-15% of the Canadian population is allergic to fungi (Miller, 1992). Whether or not people exposed to fungi develop symptoms depends on the nature of the fungal material, the amount of exposure, and the susceptibility of exposed persons (NY Dept of Health, 2000). Susceptibility varies with genetic predisposition, age, and state of health (NY Dept of Health, 2000). Fungi are frequently implicated in environments with poor indoor air quality and are associated with increased fatigue, concentration difficulties, headaches, eye, skin and throat irritation, increased frequency of common colds, wheeze and skin sensitization (Whillans, 1995). Risk of flu symptoms, diarrhea, dermatitis, malaise, epistaxia, hypersensitivity pneumonitis, and fever also increases in those exposed to mould (Health Canada, 1995). Fungal infections may also occur in the immunosuppressed (Miller, 1992).

B3. Continued

Indoor Air Quality Guidelines

Numerous authors maintain that a tolerable exposure to mould spores in indoor air ranges from 100-1000 CFU/m^3 (colony forming units). National Health & Welfare Canada suggests that up to 150 CFU/m^3 in government office buildings is acceptable if there is a mixture of species reflective of the outdoor air spores. In the summer time, 500 CFU/m^3 is acceptable if the species present are primarily tree and leaf fungi. Otherwise, a level of 50 CFU/m^3 for individual species of fungi is deemed acceptable indoors (Health Canada, 1995). Currently in Canada, no limits or standards for IAQ in schools exists.

Literature

Literature states that schools are more likely to experience IAQ deterioration due to:

- aging facilities;
- deterioration of the efficiency of the heating, ventilation and air-conditioning system;
- insufficient maintenance of schools;
- life expectancy of facilities such as portables being exceeded;
- construction of more tightly sealed buildings;
- and the use of synthetic building materials.

Description of Sample/Population

B4. Describe the numbers and type(s) of subjects to be included. If appropriate, specify the number of subjects in each study group. Provide a rationale for the sample size and include sample size calculations where appropriate.

There are a total of 217 schools within the Capital Health Region. It is commonly known in statistics that, in order for the results of an experiment to approach a normal distribution, a sample size equal to or greater than 30 must be obtained. Due to budgetary and time constraints, a sample size of 40 was chosen. To be representative of the whole school population, these 40 schools will be selected via stratification and random sampling techniques. Using parametric statistical analysis, the results from the sample will be applied to all schools within the Capital Health Region, thus allowing the investigators to answer the previously stated research questions (please refer to question B2).

The schools will be stratified into two levels:

- Geographic location
- Episodes of water damage and/or fungal growth

Stratifying the schools into geographic location allows for all schools boards to be proportionally represented within the Capital Health Region. By stratifying by episodes of water damage and/or fungal growth, it will be possible to samples in schools that have had these problems, and those that have not. Other parameters, such as age of initial construction and school building will be captured via random sampling.

Within selected schools, there will be a representative number of classrooms selected. Classes will be selected by using the results obtained from the Screening Survey (please see attachment #9). In this document, the schools will provide the different phases of construction on a schematic, which will allow for selection of rooms. Currently, the details of the experimental design are still under development with a University of Alberta statistician.

B5. List any subject inclusion/exclusion criteria.

Not Applicable. All schools within the Capital Health Region have an equal chance of being randomly selected for the study.

B6. Please check (√) if any of the subjects who will be recruited fall into one or more of the following categories:	
	Under 18 years of age
	Cognitively Impaired
	Residing in institutions (e.g. prison, extended care facility)
	Students
	Employees of researchers' organization
	Have language barriers (e.g. illiterate, not English-speaking, dysphasic)
	In another country

Description of Research Procedures

B7. Provide a summary of the design and procedures of the research. Provide details on the methods of data collection and data analysis, time commitment for the subjects etc. Please note that any and all study measures need to be appended to the copies of the research / grant proposal (e.g. questionnaire, interview guides, rating scales etc.).

Data Collection:

Questionnaires as well as visual inspection will be used to obtain information regarding the school maintenance history, central HVAC system and the room to be sampled (see attachment #10, 11, 12).

1. **Pre-Screening Survey**
   - General characterization of schools
   - Currently being used for experimental design purposes
   - Completed by school facilities personnel
   - Estimated completion time of one hour

2. **Screening Survey**
   - Provides information regarding the different phases with respect to construction type, water damage, building materials etc. This will enable the subsequent selection of rooms to be sampled.
   - The questionnaire is self applied and will be completed by the school facility personnel.
   - Estimated completion time of half an hour.

3. **School Maintenance History Survey**
   - Determine episodes of water damage and recent renovations/upgrades in the school. Maintenance of school mechanical systems will also be assessed and general health of the school population evaluated.
   - Self-applied
   - Completed by school facility personnel
   - Estimated completion time of one hour, pilot test pending

4. **Ventilation Survey**
   - Assesses the general condition of the ventilation system
   - Inspection and interview
   - Completed by field investigator with the assistance of a facility personnel and the school custodian.
   - One completed for each ventilation system
   - Estimated completion time of one hour, pilot test pending

5. **Room survey**
   - Investigate the indoor air quality of individual rooms.
   - Self-applied
   - Completed by a field investigator
   - Estimated completion time of one hour, pilot test pending

School selection:
- Experimental design will be based on a stratified sampling approach. For a summary of statistical design, please refer to B4.

Data Analysis:
- Parametric statistical analysis
- Correlate measured results (CO, CO_2, temperature, humidity, fungal count) and survey data

Procedure of Research:

Awareness campaign (pretest):

- There will be a press release one day prior to the sampling date informing the public of the study, the participating parties and a brief description of the activity that will ensue.

- Information sheet will be forwarded to parents in September and reminders will be issued for subsequent sampling rounds.

- Introductory letter and information sheet to the principal of the selected schools will be faxed by August 31, 2001. This will be followed up by a phone call to further discuss the letter and where necessary a meeting between the field investigators and the principal can be arranged to further clarify any issues. The commitment and assistance of the principal will be subsequently sought and obtained. Up to ten principals may choose not to participate without compromising the integrity of the experimental design.

Field procedures will be divided into round one (September/October), round two (December/January) and round three (February/March 2002). The activities of round two and three will be a repetition of round one in the different selected schools.

Procedures of each round:

- Upon receipt of response forms an appointment is made with participating schools. Confirmation of appointment is made by telephone one day before the sampling date.

- Field investigators arrive in selected school at prescheduled time and meet with the school contact, facilities services contact and head custodian.

- A field investigator with assistance of facilities personnel and the head custodian will complete HVAC survey.

- Sampling location is confirmed from schematic and samplers are deployed to the location inside and outside as per sampling protocol (see attachment #13)

- The visit is completed in half a day with one hour spent in inspection with the assistance of the facility personnel and a sampling period of about 20 minutes in each room.

- Samples are transported to the Provincial Public Health Laboratory for analysis.

Air sampling and analysis equipment:

The following air samplers will be used for both indoor and outdoor sampling (please see attachment #13)

- RCS plus handheld sampler with Rose Bengal Agar will be used for mould sampling.
- Q-track will be used for the measurement of CO, CO_2, temperature and relative humidity
- Velocical plus air velocity meter will be used for measurement of air flowrate

Laboratory analysis:

- Incubate sample for 7 days at 28-30°C
- Identify mould species using microscopic mount and count the mould colonies observed.

Questionnaires (attachment numbers 8, 9, 10, 11, 12):

Prescreening, Screening, School Maintenance History, HVAC, and Room Surveys

B8. Which treatments or procedures are additional to those required for standard patient care?

Not Applicable.

B9. If the procedures include a blind, under what conditions will the code be broken and what provisions have been made for this? Who will have the code?

Not Applicable.

Obtaining Consent

B10. Clearly detail who will be recruiting subjects and obtaining consent, and the procedures for doing this. If appropriate specify whether subjects will be randomly assigned to groups before or after consent has been attained.

On behalf of Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools, school superintendents have agreed to participate in this study (see attachment #15). Facilities services personnel will be asked to complete a consent form before participating in onsite HVAC investigations

(see attachment #6). In addition, school principals will be asked to complete a consent form on behalf of their schools (see attachment #4). Consent forms will be mailed to schools three weeks prior to scheduled sampling times. Schools will be randomly assigned to groups before consent has been obtained from facilities personnel and principals.
B11. Specify methods for dealing with groups identified in #B6. If the subjects are not able/competent to give fully informed consent, who will consent on their behalf? Not Applicable.
B12. If the subjects will be offered compensation for participating in the research, provide details. Specify the amount, what the compensation is for, and how payment will be determined for subjects who do not complete the study. Not Applicable.
B13. Do any of the procedures include the use of deception or partial disclosure of information to subjects? No. If yes, provide rationale for the deception or partial disclosure. Describe the procedures for (a) debriefing the subjects and (b) giving them a second opportunity to consent to participate after debriefing. Not Applicable.

Recruitment Aids/Information Letters/Consent Forms					
B14. Are you planning to use any recruitment aids such as posters, newspaper advertisements, radio announcements, or letters of invitation? Yes, information letters. If so, please indicate the reading level of each aid and check (√) if it has been attached to the form as an appendix.					
Recruitment Aid #1 – Specify (e.g. poster, letter etc.):					
√	Not Applicable		Reading Level		Attached

B.14 Cont'
Recruitment Aid #2 – Specify:

√	Not Applicable		Reading Level		Attached
Information Letter #1 – Information letter to principle.					
	Not Applicable	12.0	Reading Level	√	Attached
Information Letter #2 – Information sheet for parents.					
	Not Applicable	9.2	Reading Level	√	Attached
Consent Form #1 – Consent form for school principals					
	Not Applicable	12.0	Reading Level	√	Attached
Consent Form #2 – Consent form for facilities services personnel					
	Not Applicable	11.4	Reading Level	√	Attached

B15. What steps have been taken to make the recruitment aids, information letters, and consent forms comprehensible to the person(s) giving consent?

Information documents were reviewed by investigators, and the Steering Committee (including representatives of Capital Health Authority, Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools). Grade levels were determined using Word v7.0 Grammar check.

Risks and Benefits

B16. What are the benefits of the proposed research for the subject and/or for scientific knowledge in general?

The Capital Health Authority believes that this project will allow for enhancement of the local community's quality of life through better health for school children. Some of the community benefits include:

- Reducing the number of sick days, thereby decreasing the financial burden on the healthcare and education systems;
- Healthier children and teachers
- Proactive school IAQ management
- Improved public awareness about IAQ
- *Status of IAQ within individual schools, and overall in Capital Health Region*

B17. What adverse effects may result from the research? How will adverse effects be dealt with? Please note that adverse effects are not limited to physical risks, but include psychological, emotional, and spiritual risks as well.

There are no anticipated health or safety risks.

Privacy and Confidentiality

B18. What steps will be taken to respect the privacy of the subjects and protect confidential data?

This study will not call for personal information from students or staff. Information provided will be kept for at least five years in a secure area. All information will be held private, except when professional codes of ethics or legislation requires reporting.

B19. Identify any agencies or individuals who will have access to confidential data now or in the future.

Individuals: investigators, thesis committee
Agencies: Capital Health, Edmonton Public School Board, Elk Island School Board, Elk Island Catholic School District

B20. Do you anticipate any secondary analysis of the data? Please note that any secondary analysis requires further research ethics approval.

Yes. A research proposal by a University of Calgary graduate student is being considered for funding by the Capital Health Authority. The student would investigate possible correlations between indoor mould contamination and health concerns. As part of this study, the student might wish to do secondary analysis of data obtained in the IAQ study. An additional ethics approval would be sought prior to proceeding with this secondary analysis.

Attachments:

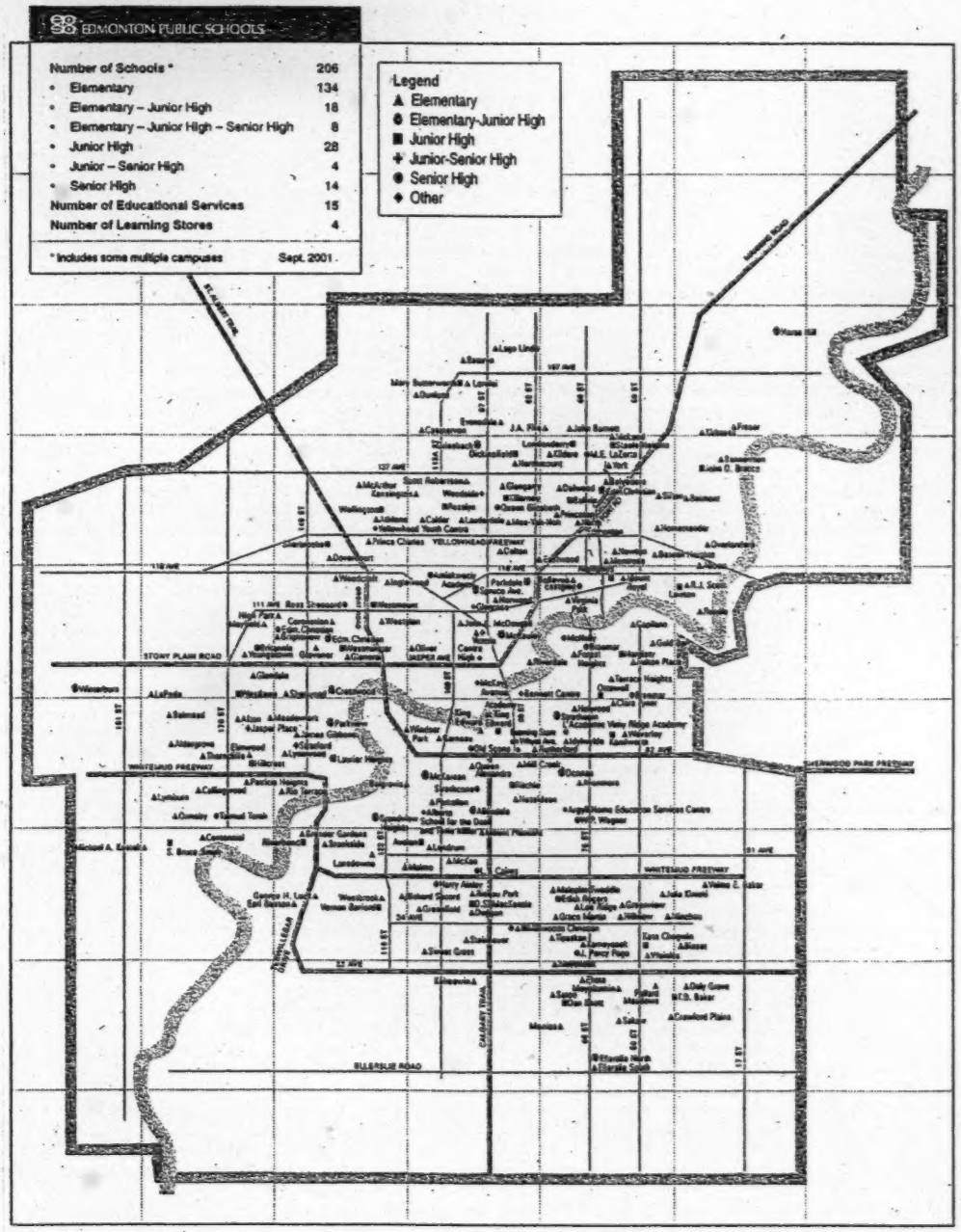
1. Proposal
2. Budgets and Letter regarding remuneration to field investigators
3. Information Letter to principal
4. Principal Response/Consent form
5. Information Letter to Director of Facilities Services
6. Facilities Services Response/Consent form
7. Information Sheet for distribution to parents
8. Pre-Screening Questionnaire
9. Screening Questionnaire
10. School Maintenance History Survey
11. HVAC Survey
12. Room Survey
13. Sampling protocol
14. Conduct protocol
15. Letters of Support

Appendix 4.1 List of Schools Within the Population

Edmonton Public Schools	Edmonton Public Schools	Edmonton Public Schools	Edmonton Public Schools	Edmonton Public Schools
Abbott	Callingwood	Elmwood	Holyrood	Lansdowne
Academy at King Edward	Capilano	Evansdale	Homesteader	LaPerle
Afton	Centennial	Forest Heights	Horse Hill	Lauderdale
Aldergrove	Center High	Fraser	Inglewood	Laurier Heights
Allendale	Clara Tyner	Fulton Place	J. A. Fife	Lawton Campus
Athlone	Coronation	Garneau	J. Percy Page	Lee Ridge
Avalon	Crawford Plains	George H. Luck	James Gibbons	Lendrum
Avonmore	Crestwood	Glendale	Jasper Place	Londonderry
Balwin	D. S. MacKenzie	Glengarry	John A. McDougall	Lorelei
Bannerman	Daly Grove	Glenora	John Barnett	Lymburn
Baturyn	Dan Knott	Gold Bar	John D. Bracco	Lynnwood
Beacon Heights	Delton	Grace Martin	Julia Kiniski	M. E. LaZerte
Belgravia	Delwood	Grandview Heights	Kameyosek	Malcolm Tweddle
Bellevue	Dickinsfield	Greenfield	Kate Chegwin	Malmo
Belmead	Donnan	Greenview	Keheewin	Mary Butterworth
Belmont	Dovercourt	Griesbach	Kenilworth	Mayfield
Belvedere	Duggan	Grovenor	Kensington	McArthur
Bisset	Dunluce	Hardisty	Kildare	McCauley
Brander Gardens	Earl Buxton	Harry Ainlay	Killarney	McKee
Brightview	Eastglen	Hazeldean	King Edward	McKernan
Britannia	Eastwood	High Park	Kirkness	McLeod
Brookside	Edith Rogers	Highlands	L. Y. Cairns	McNally
Caernarvon	Ekota	Hillcrest	L'Académie Vimy Ridge	Meadowlark
Calder	Ellerslie Campus	Hillview	Lago Lindo	Mee-Yah-Noh

Edmonton Public Schools	Edmonton Public Schools	Edmonton Public Schools	Elk Island Catholic Schools	Elk Island Public Schools
Menisa	R. J. Scott	Thorncliffe	Arch Bishop Jordan	Ardrossan Elementary
Meyokumin	Richard Secord	Tipaskan	Father Kenneth Kearns	Ardrossan Jr/Sr High
Meyonohk	Rideau Park	Velma E. Baker	Holy Redeermer	Bev Facey Community
Michael A. Kostek	Rio Terrace	Vernon Barford	Jean Vanier	Brentwood
Minchau	Ritchie	Virginia Park	Madonna	Campbelltown
Montrose	Riverbend	W. P. Wagner	Our Lady of Perpetual Help	Clover Bar
Mount Pleasant	Riverdale	Waverley	St. Luke	Colchester
Mount Royal	Ross Sheppard	Weinlos	St. Theresa	F.R. Haythorne
Newton	Rosslyn	Wellington		Fultonvale
North Edmonton	Rundle	Westbrook		Glen Allen
Northmount	Rutherford / Idylwylde	Westglen		Mills Haven
Norwood	S. Bruce Smith	Westlawn		Ministik
Old Scona Academic	Sakaw	Westminster		Pine Street
Oliver	Satoo	Westmount		Salisbury
Ormsby	Scott Robertson	Windsor Park		Sherwood Heights
Ottewell	Sherbrooke	Winterburn		Uncas
Overlanders	Sherwood	Woodcroft		Wes Hosford
Parkallen	Sifton	York		Westboro
Parkdale	Spruce Avenue	Youngstown		Woodbridge Farms
Parkview	Steele Heights			Wye
Patricia Heights	Steinhauer			
Pollard Meadows	Strathcona			
Prince Charles	T. D. Baker			
Princeton	Talmud Torah			
Queen Elizabeth	Terrace Heights			

Appendix 4.2 Map of Edmonton Public Schools



Appendix 5. Pre-Screening Survey

Title of Project: *Indoor Air Quality in Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools.*

Principal Investigators:

Steven Probert
Capital Health
(780) 413-7933

Warren Kindzierski
University of Alberta
(780) 492-0247

Co-Investigators:

Lisa Johnston
EPS
(780) 429-8132

Dale Lechelt
EICS
(780) 417-8125

Paul Steinhubl
EICS
(780) 467-8896

Background Information:

Capital Health and the University are investigating the quality of indoor air within public schools. The Project will measure certain indoor air quality parameters (fungal aerosols and ventilation rates) and determine building designs and conditions that may contribute to indoor air quality. The results of this questionnaire will assist the investigators in selecting a representative sample of your schools.

Instructions:

This questionnaire is to be completed by a qualified person designated by your school board's co-investigator, as listed above. Please answer all of the questions by printing in the spaces provided or placing a checkmark in the correct box. Please contact your co-investigator or Steven Probert for assistance. The completed questionnaire is to be returned to your co-investigator **by May 23, 2001.**

School Name: _____

Street Address: _____ Postal Code: _____

Circle City/County: Edmonton Sherwood Park Strathcona County

Who completed this questionnaire?

Name: _____ Phone No.: _____

1. What category does the school fall into?

   K-6: ☐ K-9: ☐ K-12: ☐ 6-9: ☐ 6-12: ☐ 9-12: ☐

2. What is school utilization rate based on the 2001 Alberta Infrastructure guideline?

   _____(%)

3. What type of foundation(s) are found in school excluding portables/pods? (please check all that apply)

   Basement: ☐ Slab-On-Grade: ☐ Crawl Space: ☐

4. What year did the initial construction of school take place? _____

5. Are portables and/or pods present?

   Yes: ☐ No: ☐

6. What type of system(s) are used to heat the school? (check all that apply)

   Forced Air: ☐ Hot Water/Steam: ☐ Other: ☐

7. Has the school ever had a modernization (as defined in the 1997 draft of the School Capital Plan) and/or a heating and ventilation system upgrade?

   Yes: ☐ No: ☐

8. In the past 5 years, has the school had a history of water damage (e.g. roof leak, plumbing leak, poor surface drainage around buildings)?

   Yes: ☐ No: ☐

9. In the past 5 years, has the school had an investigation of IAQ concerns?

   Yes: ☐ No: ☐

10. In the past 5 years, has the school ever had to take actions to resolve fungal (eg. mold, mildew) contamination concerns?

    Yes: ☐ No: ☐

School Board	5. Portables /pods	6. Heating Systems	7. Modernization	8. Water damage	9. IAQ concerns	10. Action against fungi
EICS (8 total)	1 no 7 yes	3 forced air & hot water 2 hot water 3 forced air	6 no 2 yes	7 no 1 yes	5 no 3 yes	8 no 0 yes
EIPS (20 total)	9 no 11 yes	12 forced air & hot water 3 hot water 5 forced air	19 no 1 yes	6 no 14 yes	17 no 3 yes	18 no 2 yes
EPS (189 total)	112 no 77 yes	3 no 186 yes	155 no 34 yes	165 no 24 yes	105 no 84 yes	157 no 32 yes
SUMMARY 217 schools	122 no 95 yes	15 forced air & hot water 5 hot water 8 forced air - 186 forced air in EPS 3 not forced air in EPS	180 no 37 yes	178 no 39 yes	127 no 90 yes	183 no 34 yes

Appendix 6. Pre-Screening Survey Results

School Board	District	1. Grades	2. Utilization rate (%)	3. Type of foundation	4. Year of initial construction
EICS (8 total)	6 Sherwood Park 2 Strathcona County	7 are K-9 1 is 9-12	max 130.6 min 74.0 avg 102.0	4 slab-on-grade & crawl space 4 slab-on-grade	3 in 60s 3 in 70s 2 in 80s
EIPS (20 total)	13 Sherwood Park 7 Strathcona County	14 are K-6 3 are 7-9 1 is 7-12 2 are 9-12	max 121.7 min 59.3 avg 89.2	3 slab-on-grade & crawl space 16 slab-on-grade 1 basement & slab-on-grade & crawl	7 in 50s 3 in 60s 8 in 70s 1 in 80s 1 in 90s
EPS (189 total)	Edmonton	129 are K-6 30 are 7-9 12 are 10-12 16 are K-9 1 is K-12 1 is 6-12	max 169.2 min 23.9 avg 70.8	105 slab-on-grade 64 crawl space 19 basement	2 in 00s 7 in 10s 5 in 20s 1 in 30s 12 in 40s 56 in 50s 39 in 60s 29 in 70s 25 in 80s 12 in 90s
SUMMARY 217 schools	19 Sherwood Park 9 Strathcona County 189 Edmonton	143 are K-6 33 are 7-9 12 are 10-12 23 are K-9 1 is K-12 1 is 6-12 1 is 7-12 3 are 9-12	max 169.2 min 23.9	125 slab-on-grade 64 crawl space 19 basement 7 slab-on-grade & crawl space 1 basement & slab-on-grade & crawl	2 in 00s 7 in 10s 5 in 20s 1 in 30s 12 in 40s 63 in 50s 45 in 60s 40 in 70s 28 in 80s 13 in 90s

Appendix 7. Letter to the Principal

September 15, 2001

Dear Principal,

RE: ***Indoor Air Quality in Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools***

The World Health Organization and international environmental health agencies recognize indoor air pollution as a serious threat to human health. In addition, it is recognized that children are a more susceptible sub-population to air pollutants, and that indoor air quality (IAQ) within schools affects both student health and performance.

As a result, Capital Health and the University of Alberta are conducting a new IAQ study in which your school has been randomly selected to participate. The purpose of this study is to determine the status of IAQ in schools, to develop IAQ investigative techniques and IAQ management strategies. This study has received the support of your Superintendent but also requires your consent for this school to participate in the study.

This study will involve a physical inspection of selected rooms and ventilation systems, and monitor important IAQ parameters. In order to obtain representative results, it is essential that sampling occur under normal school operating conditions. This study will require one or two visits to your school in the fall and/or winter with the visits lasting 4 to 6 hours. The attached *Information Sheet* and *Conduct Protocol* contain additional information including procedures to be followed by field investigators within the school.

The field investigators will report the results of this study in several different forms. There will be an immediate verbal report of any imminently dangerous conditions and a preliminary written report of the results after each school visit. Lastly, there will also be a final comprehensive report scheduled for completion in summer 2002. These reports will be provided to the school co-investigator, identified on the *Information Sheet*, for distribution to the appropriate school staff.

Otherwise, the results of this study will be kept confidential by the field investigators.

As a participating school, the investigators require your assistance in informing people about the study and in scheduling the aforementioned school visits. If you wish to participate, please complete and return the attached *Response/Consent Form* **by September 12, 2001.** In order to inform staff, parents and students, the investigators would like you to distribute the *Information Sheet* as an attachment to your school newsletter.

If you have any questions or concerns, please contact your school co-investigator. Thank you for your co-operation.

Yours truly,

_____

Dr. Warren Kindzierski, PhD., PEng.
Principal Investigator
University of Alberta

_____

Dr. Gerald Predy, MD., FRCPC
Medical Officer of Health
Capital Health

Attachments: *Response/Consent Form*
Information Sheet
Conduct Protocol

Appendix 8. Information Sheet

Environmental Health Services

Civil and Environmental Engineering

Indoor Air Quality in Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools

What is the purpose of the project?

The World Health Organization and international environmental health agencies recognize indoor air pollution as a serious threat to human health. In addition, it is recognized that children are a more susceptible sub-population to air pollutants, and that indoor air quality (IAQ) within schools affects both student health and performance.

As a result, Capital Health and the University of Alberta are conducting an exciting new indoor air quality study within Edmonton and Strathcona County. The purpose of this project is to determine the status of IAQ in schools, to develop IAQ investigative techniques and IAQ management strategies.

What data will be collected?

This study includes IAQ monitoring and a physical inspection of the school. The IAQ parameters monitored include carbon dioxide, carbon monoxide, temperature, humidity, mould, and ventilation rates.

The physical inspection of selected rooms will include source identification of indoor air pollutants, and a determination of the status of ventilation systems serving those rooms.

How will this information be used?

This information will be used to develop more effective IAQ investigative and management strategies. It will allow further development and validation of methods for IAQ monitoring and surveying. It will allow school boards and government to develop 'state of the art' IAQ management strategies.

When will the study be conducted?

This study has several stages including study design, data collection, data analysis and reporting. The study began in January 2001 and requires about 18 months to complete. The schools that consent to participate in the study will receive confirmation in September 2001. The data collection is scheduled from September 2001 to March 2002. Lastly, data analysis and reporting will be done.

The sampling must occur during normal school hours for results to be representative of student exposure. There will be one visit to most schools but there are a few schools needed for two seasonal visits. Based upon school differences, each school visit will require 4 to 6 hours.

How will this study affect the school?

The study is designed to minimize classroom disruption. It is conducted during normal school hours requiring air sampling and inspection in occupied rooms. However, Capital Health designed it after a pilot study (1999/2000) that was successful in minimizing classroom disruption.

In addition to Principal consent, the study requires the patience and support of school staff, and assistance in scheduling the visits and distributing *Information Sheets*.

The study does not require any assistance or personal information from parents or students.

What are the benefits of this study?

- Improved IAQ investigative methods and management strategies
- School IAQ baseline levels
- Increased public awareness of IAQ
- Healthier staff and students
- Improved learning environments

Who is conducting the study?

Capital Health is conducting the study in cooperation with Edmonton Public Schools, Elk Island Public Schools, and Elk Island Catholic Schools.

The three field investigators identified below are graduate students from the University of Alberta.

How will the information be reported?

The results of this study will be available to the public in a comprehensive report scheduled for release in summer 2002.

The identity of individual schools will be released to the respective school boards and Capital Health, but otherwise the identity will be kept confidential by the investigators.

Where can I get more information?

Steven Probert
Principal Investigator
Project Manager
Capital Health
(780) 413-7933

Warren Kindzierski
Principal Investigator
Project Consultant
University of Alberta
(780) 492-0247

Lisa Johnston
Edmonton Public Schools
Co-Investigator
(780) 429-8132

Dale Lechelt
Elk Island Public Schools
Co-Investigator
(780) 417-8125

Arjen DeVries
Elk Island Catholic Schools
Co-Investigator
(780) 991-1340

Lorelei Betke
Field Investigator
University of Alberta
(780) 492-8548
lbetke@ualberta.ca

Corinne Stocco
Field Investigator
University of Alberta
(780) 492-8548
cstocco@ualberta.ca

Mary Unobe
Field Investigator
University of Alberta
(780) 492-8548
munobe@ualberta.ca

Appendix 9. Response / Consent Form

Title of Project: *Indoor Air Quality in Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools.*

Principal Investigators:

Steven Probert
Capital Health
(780) 413-7933

Warren Kindzierski
University of Alberta
(780) 492-0247

Co-Investigators:

Lisa Johnston
EPS
(780) 429-8132

Dale Lechelt
EICS
(780) 417-8125

Arjen DeVries
EICS
(780) 991-1340

Field Investigators:

Lorelei Betke, Corinne Stocco and Mary Unobe
University of Alberta
(780) 492-8548 or cstocco@ualberta.ca

Instructions:

The requested information will provide the principal's consent to participate in the study and enable the investigators to schedule their visit(s). If you need additional information to assist you in making this decision, please call your school board's co-investigator or Capital Health's principal investigator identified above. **Please complete and return this form via facsimile by September 12, 2001 to:**

School IAQ Study
Warren Kindzierski
University of Alberta
FAX # (780) 492-8289

After receiving the completed form, the investigators will contact you to schedule their visit(s). Thank-you for your co-operation.

School Name: _____
Street Address: _____ Postal Code: _____
Phone No.: _____ Fax No.: _____

1. If you agree to participate, please read the below paragraph and indicate your consent by signing below.

I understand that by signing below, my school will be participating in the indoor air quality investigation that is being completed by Capital Health and the University of Alberta. I know that my school is in no way obligated to participate, and that I have the right to withdraw at any time during the study, without consequence. I have received and read a copy of the information sheet, and have had the opportunity to ask an investigator my questions. I also understand that all data that will be obtained during this study will be kept confidential and will be kept for at least five years in a secure area. I also know that it is possible that the data obtained from the investigation may be looked at again in the future to help answer other study questions.

I agree to allow this school to participate in the study:

_____ _____

Principal's Name Date

_____

Principal's Signature

2. In order to investigate seasonal variability, the investigators may like to schedule a visit to your school in both Fall 2001 (September/October) and Winter 2002 (January/ February/March). Please indicate below if you support two visits to your school:

Yes No

3. In order to facilitate scheduling, please attach a school calendar indicating any holidays, PD Days and other days in which 'normal' room activities may not be occurring in the sample locations (e.g., fieldtrips, school assemblies). If this calendar is not yet available, please fax the response form without it, and the investigators will be as flexible as possible during scheduling.

4. In order to facilitate HVAC investigations, please provide the following information:

Head Custodian's Name _____

Head Custodian's Phone Number _____

5. Please list any questions, comments or concerns:

_____

Appendix 10. Conduct Protocol

Title of Project: *Indoor Air Quality in Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools.*

PRIOR TO SCHOOL VISIT

By mid-September, mail the following to selected schools:

Principal Information Letter
Information Sheet
Response/Consent form
Conduct Protocol

In early October, complete the following tasks:

Phone call to set up sampling appointment with school (Note: For EPSB schools, no calls to principals before 10:30 am)
Establish school contact person (Principal or designate)
Establish facilities contact person with each project co-investigator (Dale, Lisa, Arjen)
Outline time requirements
Outline sampling procedure
Finalize school visits schedule

DAY BEFORE SCHOOL VISIT

Phone call to school's contact person to confirm visit (For EPSB schools, no calls before 10:30 am if contact person is principal).
Phone call to facilities services' contact person to confirm meeting time and place for ventilation survey

DAY OF SCHOOL VISIT

Upon Arrival

Ensure visible placement of identification tags
Sign in at main office
Introduce self to school personnel and/or designate
Meet up with facilities contact person
Explanation of visit to facilities contact person

Ventilation Survey

Inspect the ventilation system and complete survey with assistance of facilities contact person

Room Survey

In keeping with the *EPSB Blueprints Program,* begin Room Surveys anytime after 10:30am
Introduce self to teacher/staff member in each room to be sampled and outline sampling procedure. If requested, briefly explain purpose of study
Set up equipment while minimizing room disruption
Sampling to be conducted as per *Sampling Protocol*
Remove equipment and quietly exit room

Outdoor Sampling

Access appropriate outdoor air intakes
Sampling to be conducted as per sampling protocol

Leaving School

Sign out at main office, and thank contact person for time/assistance
Advise school administrator, Capital Health and school board's co-investigator of any imminent nature

AFTER VISIT

Provide school co-investigators with preliminary sampling results as they become available for each school.
Distribute thank-you notes to schools that participated

Appendix 11. School Screening Survey

Title of Project:	*Indoor Air Quality in Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools.*	
Principal Investigators:	Steven Probert Capital Health (780) 413-7933	Warren Kindzierski University of Alberta (780) 492-0247
Co-Investigators:	Lisa Johnston EPS (780) 429-8132	Dale Lechelt EIPS (780) 417-8125
	Arjen DeVries EICS (780) 467-8896	
Field Investigators:	Lorelei Betke, Corinne Stocco and Mary Unobe University of Alberta (780) 492-8548 or (cstocco@ualberta.ca)	

Instructions:

This survey will enable investigators to select rooms in your school for sampling of indoor air quality parameters. If a question is unclear, please refer to the glossary of terms or contact one of the field investigators for assistance. Thank-you for your co-operation.

This survey is to be completed by a qualified person appointed by the school board co-investigators listed above. The completed survey is to be **submitted to your co-investigator by August 24th, 2001.**

Please begin by obtaining a schematic of the school floor plan that identifies all rooms by number and function. This schematic should be stapled to the back of this survey. Outline each 'construction phase' in red and identify it as Phase I, II, III, IV, etc. Each portable or pod should be identified as a separate 'construction phase'. Rooms with a history of water damage and/or visible or suspected fungal growth should be indicated with an asterisk (*), as shown in the sample schematic.

Please complete the attached Screening Survey for each 'construction phase', answering all questions by placing a check mark or appropriate number in the correct box(es). If there is more than one correct box, check all that apply.

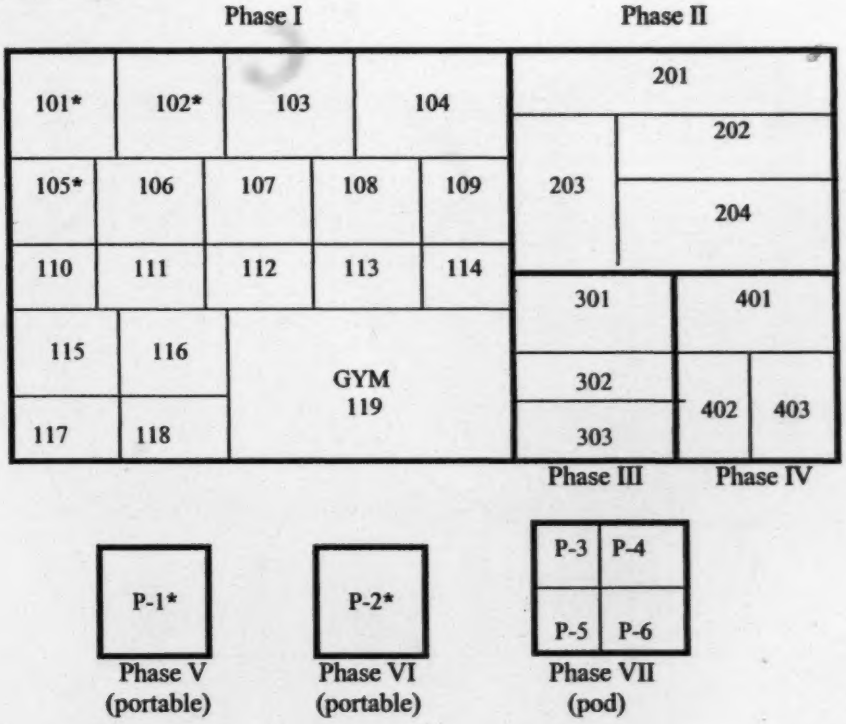
Who completed this questionnaire?

Name: _____ Phone No.: _____

Name: _____ Phone No.: _____

Date of completion (dd/mm/yy): _____

Sample schematic



* Denotes rooms that have had fungal investigation and/or water damage.

Glossary of terms for the **Screening Survey**

Term	Definition
Construction Phase	A stage in the development of the project that occurs after the construction contract has been awarded and until the certificate of substantial completion has been issued.*
HVAC System	These are systems that service multiple rooms and exclude unit serving a single room (eg. unit ventilator, small forced-aired furnace in a pod or portable). Please use the school's identifier, or room numbers that they service.
Masonry	Stone-work, other than brick.
Permanent	The main school building structure, that cannot easily be relocated.
Pod	This is typically one unit that has several classrooms within it. It may or may not be connected to the permanent building structure via hallways.
Portable	This is typically a one classroom unit which has its own HVAC system. This structure may or may not be attached to the permanent building structure via hallways, and can be relocated to other locations.
Suspected mould growth	Musty smells.
Visible mould growth	>0.5 square foot of mould For example: ceiling tiles, ductwork, wallboard panels.
Water damage	Damage that occurs repeatedly. For example: roof leak, water main break, and surface drainage leak.

* Based on the 2001 Alberta Infrastructure Guidelines

Appendix 12. School Maintenance History Survey

Title of Project:	*Indoor Air Quality in Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools.*	
Principal Investigators:	Steven Probert Capital Health (780) 413-7933	Warren Kindzierski University of Alberta (780) 492-0247
Co-Investigators:	Lisa Johnston EPS (780) 429-8132	Dale Lechelt EIPS (780) 417-8125
	Arjen DeVries EICS (780) 467-8896	
Field Investigators:	Lorelei Betke, Corinne Stocco and Mary Unobe University of Alberta (780) 492-8548 lbetke@ualberta.ca	

Instructions:

The purpose of this questionnaire is to determine episodes of water damage and recent renovations/upgrades in the school. Maintenance of school mechanical systems will also be assessed and incidence of air quality complaints evaluated. This will assist investigators in the interpretation of sampling results gathered from the various locations in the school.

This school maintenance history survey is to be completed for selected schools by a facilities personnel offsite. It may be most convenient to complete this survey in conjunction with the School Screening Survey since both are completed offsite and are looking for related information.

There are four main questions in this survey, as well as a schematic of the school floor plan that has been included for reference purposes. Please answer all questions by printing in the appropriate boxes. If a question is unclear, please contact a field investigator for assistance. Return to investigators by September 15. Thank-you for your cooperation.

SCHOOL NAME _____

Who completed this questionnaire? Name: _____

Phone No.: _____

A. School Maintenance History:

1. Complete the table with any incidents of major water damage that occurred within the past five years:

Incident	Phase Number(s)	Room Number(s)	Year of Incident	Was incident resolved? (Yes/No/N/A)	Year Resolved
Roof Leak					
Sewer or Water Main Break					
Surface Drainage Leak					
Other (please describe)					
_____					
_____					

2. Indicate if the following renovations/upgrades occurred within the past five years:

Renovation/Upgrade	Year of work	Phase Number(s)	Room Number(s)
Adjustment to Room Size			
Open Room Enclosed			
Room Conversion			
Other (please describe)			
_____			
_____			
_____			

3. Please answer the following set of questions:

a) How often is the HVAC system balanced?

On a routine basis: ☐ As needed: ☐

b) Is there a preventative maintenance program for the HVAC system?

Yes: ☐ No: ☐

If so, is there a method to assess the effectiveness of the HVAC maintenance program?

Yes: ☐ No: ☐

c) Are the air ducts professionally cleaned on a routine basis?

Yes: ☐ No: ☐ Unknown: ☐

d) When were the air ducts last professionally cleaned?

2001 ☐ 2000 ☐ 1999 ☐ Unknown ☐ Other ☐

If other, indicate year of cleaning _____

4. Please answer the following questions:

a) In the past five years, have the school staff, parents or students reported any concerns about the school's indoor air quality?

Yes ☐ No ☐

b) In the past year, approximately how many of these individuals have reported indoor air quality concerns?

1-10 ☐ 11-30 ☐ 31-50 ☐ >50 ☐

Glossary of terms for the **School Maintenance History Survey**

Term	Definition
Air balancing	The process of measuring the distribution of air supply and the amounts of exhausted air.
Ducts	Conduits through which supply air travels.
Heating, ventilation and air conditioning (HVAC)	Air handling systems designed primarily for temperature, humidity, odour control, and air quality.
Major Water Damage	Damage that occurs repeatedly
Preventative maintenance	Pro-active steps to prevent problems for the HVAC system (eg. cleaning schedule).

Appendix 13. Ventilation Survey

Title of Project: *Indoor Air Quality in Edmonton Public Schools, Elk Island Public School and Elk Island Catholic Schools.*

Principal Investigators:

Steven Probert
Capital Health
(780) 413-7933

Warren Kindzierski
University of Alberta
(780) 492-0247

Co-Investigators:

Lisa Johnston
EPS
(780) 429-8132

Dale Lechelt
EIPS
(780) 417-8125

Steve Hayes
EICS
(780) 467-8896

Field Investigators:

Lorelei Betke, Corinne Stocco and Mary Unobe
University of Alberta
(780) 492-8548 or munobe@ualberta.ca

Instructions:

The survey results determine information about the ventilation system in this school that may affect indoor air quality. The field investigators will complete this survey with the assistance of facility personnel as appointed by your school board's co-investigator. **Please begin by identifying the location and system label/identifier on the schematic as well as the room being serviced.** Please answer all questions by printing or placing a check-mark in the correct boxes or columns provided. If there is more than one correct box, check all that apply.

Note: This survey is for accessible portions of the air-handling units to be sampled.

School Name: _____

Street Address: _____ Postal Code: _____

Circle City/County: Edmonton Sherwood Park Strathcona County

Who completed this form:

Name: _____ Phone No.: _____

1. Answer the following questions regarding the air handling system identified below:

Air Handling System ID	
Type of air-handling system, if not HVAC:	Hot water boiler: ☐ Steam boiler: ☐ Furnace: ☐ Forced Air: ☐ Unit ventilator: ☐ Roof-top: ☐
Phase (s) and/or room (s) serviced	
Function	Heating: ☐ Humidifying: ☐ Cooling: ☐ Dehumidifying: ☐ Other: ☐

2. Description and condition of heating and cooling coils, humidifiers and drain pans:

Description	Heating Coils	Cooling Coils	Drain Pan	Reheat Coil
Clean				
Slime or mould buildup*				
Clogged			--	
Other _____				
Not accessible				
Doors well-sealed?	Yes ☐ No ☐	Yes ☐ No ☐	--	--

*take tape sample

3. Questions pertaining to indoor / outdoor air mixing:

Appearance of main mixing box	Clean ☐ Dry ☐ Dirty ☐ Wet ☐ No access ☐
Control of mixing box	Pneumatic ☐ DDC ☐ Manual ☐ Self-contained ☐ Electric ☐ Electronic ☐
Indoor : Outdoor mixing ratio	:
Outdoor delivery at all times?	Yes ☐ No ☐

4. Filter type and condition:

Dual stage filter?	Yes ☐	No ☐			
Filter type	Box ☐	Bag ☐	Roll ☐	HEPA ☐	Other ☐
Condition of filter	Clean ☐	Build up ☐	Clogged ☐		
Tight fitting filter?	Yes ☐	No ☐			
Magnehelix reading	< 1 ☐	1 – 1.5 ☐	>1.5 ☐		

5. Description of outdoor air intake(s) (OAI) & surrounding areas.

Obstructed?	Yes ☐	No ☐
Bird screen ?	Yes ☐	No ☐
Insect screen?	Yes ☐	No ☐
Sources of CO?	Yes ☐	No ☐
Protection from rain/snow?	Yes ☐	No ☐
Evidence of standing water?	Yes ☐	No ☐
Sources of contamination?	Yes ☐	No ☐
Distance between exhaust and intake	1-10 ft ☐	>10 ft ☐

6. General condition of the system:

Water collecting anywhere except the drain pan?	Yes ☐	No ☐			
Complete air handling shutdown?	Evenings ☐	Weekends ☐	N/A ☐		
Partial air handling shutdown?	Evenings ☐	Weekends ☐	N/A ☐		
Self-regulated?	Yes ☐	No ☐			
Mechanical room cleanliness	Chemical storage	Yes ☐	No ☐		
	Clean	Yes ☐	No ☐		
Supply fan operating?*	Yes ☐	No ☐	Unknown ☐		
Exhaust fan operating?*	Yes ☐	No ☐	Unknown ☐		

*Determine using smoke tube

Comments:

_____

_____

Glossary of terms for the **Ventilation Survey**

Term	Definition
Bag filter	A series of bags that blow out downstream, held in place by a frame mounted across the duct cross section.
Box filter	
Build-up	Some parts of filter mesh are visible.
Clean	Free of debris, spills, dirt and dust.
Coils	Heating and cooling heat exchangers which either add or remove heat from the air in an HVAC system.
Complete matting	Complete disappearance of mesh screen.
Construction Phase	A stage in the development of the project that occurs after the construction contract has been awarded and until the certificate of substantial completion has been issued.*
DDC	Direct Digital Control
Ducts	Conduits through which supply air travels.
Heating, ventilation and air conditioning (HVAC)	Air handling systems designed primarily for temperature, humidity, odour control, and air quality. Can be a central HVAC that services more than one room, or can be a unit ventilator.
HEPA filter	High-Efficiency Particulate Arrestance: a filter with a deep bed of randomly positioned micro glass fibers, in which the total bed depth (thickness of media) is very large in comparison to the average fiber diameter and effective pore of free path cross section.
HVAC ID	In-house label for the individual HVAC systems (eg. LDS-1).
Obstructed	Object that prevents required air flow rate through OAI.
Outdoor air intake (OAI)	Location where outdoor air is drawn into HVAC system.
Roll filter	A spool of thin, low-efficiency filter medium that traverses slowly across the filter housing, thereby moving the dirty portion to a second spool and introducing clean filter medium at the other end.
Sources of contamination	Feathers, droppings, organic matter, flue or sewer gas.
Tight fitting filter	No bypass around the filter.
Well-sealed	Free of whistling and rushing of air.

* Based on Alberta Infrastructure 2001 Guidelines

Appendix 14. Room Survey

Title of Project: *Indoor Air Quality in Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools.*

Principal Investigators:

Steven Probert
Capital Health
(780) 413-7933

Warren Kindzierski
University of Alberta
(780) 492-0247

Co-Investigators:

Lisa Johnston
EPS
(780) 429-8132

Dale Lechelt
EIPS
(780) 417-8125

Arjen DeVries
EICS
(780) 991-1340

Field Investigators:

Lorelei Betke, Corinne Stocco and Mary Unobe
University of Alberta
(780) 492-8548 or lbetke@ualberta.ca

Instructions:

The purpose of this survey is to document conditions in the rooms selected for sampling in order to assist with the interpretation of results.

Please begin by placing a check mark in the boxes provided; if more than one option is valid, check all that apply. For additional comments or schematic, please feel free to attach an extra sheet. This survey is to be completed by a field investigator who will also have a schematic of the school. One survey form is to be completed for each room to be sampled.

School Name: _____

Street Address: _____ Postal Code: _____

Circle City/County: Edmonton Sherwood Park Strathcona County

Who completed this questionnaire?

Name: _____

Date (dd/mm/yy): _____ Time: _____

	Survey Information	Comments
	General information	
1a	Construction phase	
1b	Room Number	
2	Room volume (ft^3)	V = L * W * H =
3	Number of occupants in the room (other than investigators):	0 ☐ 1-10 ☐ 11-20 ☐ 1-30 ☐ >31 ☐
4	Function of the room?	Classroom ☐ Food Studies ☐ Lab ☐ Other ☐
5	Level of room activity	Low ☐ High ☐ None ☐
6	Is there a noticeable odour?	Yes ☐ No ☐
7	List number of plants in the room	
8a	Is there a carpet in the room?	Yes ☐ No ☐
8b	If yes, describe its condition	Good ☐ Worn ☐ Dirty ☐ Other ☐
8c	Estimate area of coverage	< 100 ft^2 ☐ 100 – 200 ft^2 ☐ > 200 ft^2 ☐
	Heating and Ventilation	
9	Sources of room ventilation	Central HVAC: Yes ☐ No ☐ Windows: Open ☐ Closed ☐ N/A ☐ Doors: Open ☐ Closed ☐ Unit Ventilator: On ☐ Off ☐ Auto ☐ N/A ☐ Local Exhaust: On ☐ Off ☐ Auto ☐ N/A ☐
10a	Is there an outdoor air intake?	Yes ☐ No ☐
10b	Condition of OAI?	Clean ☐ Partially matted ☐ Clogged ☐ N/A ☐
	Potential Sources of Contamination	
11a	Is there a portable humidifier?	Yes ☐ No ☐
11b	Condition of portable humidifier	Clean ☐ Mineral Buildup ☐ Mould Growth ☐ Other ☐
12	Air freshener present?	Yes ☐ No ☐
13a	Any pets in the classroom?	Yes ☐ No ☐
13b	If yes describe	
14	Is the classroom dusty?	Yes ☐ No ☐ Somewhat ☐
15	Are there sources of CO_2 other than human breath?	Yes ☐ No ☐ Describe

16. Using the legend, complete the following table for evidence of water damage:

LEGEND→ *A: < 1 square foot , B: 1 – 10 square feet C: > 10 square feet*

Area	Water Evidence (✓)				Area Type and Size (A, B, C)				Visible Mould (✓)	Sample taken (✓)
	Dry Stain	Wet Stain	Conden-sation	Ponding	Ceiling	Floor	Wall	Window		
1										
2										
3										
4										
5										

17	Indicate ventilation sources (Provide schematic) CW = closed window OW = open window CD = closed door OD = open door FH = fume hood H = heater	
18	Additional comments	

Glossary of terms for the **Room Survey**

Term	Definition
Construction Phase	A stage in the development of the project that occurs after the construction contract has been awarded and until the certificate of substantial completion has been issued.*
Occupancy	Full time occupancy is used as a measure of space utilization. Enrollment is calculated on the number of student spaces occupied throughout the school day.*
Ponding	Pooling of water on floor.
Low activity	Little movement in room; period of lecture or minimal motion.
High activity	Elevated amounts of movement in room; cleaning by maintenance staff or physical recreation.

* Based on Alberta Infrastructure 2001 Guidelines

Appendix 15. Sampling Protocol

Title of Project:	*Indoor Air Quality in Edmonton Public Schools, Elk Island Public Schools and Elk Island Catholic Schools.*	
Principal Investigators:	Steven Probert Capital Health (780) 413-7933	Warren Kindzierski University of Alberta (780) 492-0247
Co-Investigators:	Lisa Johnston EPS (780) 429-8132	Dale Lechelt EIPS (780) 417-8125
	Arjen DeVries EICS (780) 991-1340	
Field Investigators:	Lorelei Betke, Corinne Stocco and Mary Unobe University of Alberta (780) 492-8548 or munobe@ualberta.ca	

Introduction

This section outlines the general field investigation and sampling protocols. Specific details regarding instrument calibration and sampling procedures are contained in the standard operating procedures for each instrument. Three instruments, namely the RCS Plus fungal sampler, the Q-Trak air quality meter and the Velocical Plus air velocity meter will be used for sampling. The RCS Plus will be used to establish baseline fungal levels within sampling locations. 6-minute samples will be taken and then analyzed at the Provincial Mycological Laboratory. The Q-Trak will be used to measure CO_2, CO, relative humidity and temperature. Values of CO_2 obtained will be used for comparative analysis with established standard such as ASHRAE. Ventilation rates can also be determined from CO_2 decay measurements. The Velocicalc Plus will be used to establish air flowrate measurements into the OAI as well air velocity from the diffusers located in the classrooms. Outdoor measurements with all three instruments will serve as a control for the indoor measurements in order to confirm the indoor air quality of the rooms.

GENERAL

1.0 Documentation

1.1 If possible, photograph rooms being sampled and areas of noticeable fungal contamination for reference and documentation purposes.

1.2 When completing the room survey, note number of room occupants, and describe potential interferences to air sampling and where they are located (eg. distance of heat sources or occupants to instruments).

1.3 Store all records in a secure location (pictures, Q-Trak measurements, surveys).

2.0 QA/QC Procedures and Calibration

2.1 Sample during normal classroom hours.

2.2 Calibrate Q-Trak, and RCS Plus prior to each sampling round according to instrument manuals. Velocicalc Plus has been factory calibrated.

2.3 Conduct one field blank per week for fungal samples only. Collect field blanks by placing the agar strip in the RCS Plus and immediately removing it for laboratory analysis. The RCS Plus sampler must not be operational during this procedure.

2.4 Conduct one replicate RCS Plus sample per school.

2.5 At Toxcon, verify if the RCS Plus instrument is contaminated by taking a sample in a sterile environment. If contaminated, immerse impeller in isopropyl alcohol overnight. Allow to air dry and recalibrate instrument.

2.6 Include Chain of Custody forms with all fungal samples submitted for laboratory analysis.

2.7 Store Rose Bengal Agar (RBA) strips in a clean refrigerator. Keep strips away from direct heat or sunlight. RBA strips have a shelf life of 2 months.

INDOOR INVESTIGATION AND SAMPLING PROTOCOL

Fill out IAQ advisory sheet and submit to co-investigators if the ASHRAE threshold levels for CO_2 are exceeded as well as if potential hazardous conditions exist. Furthermore, fill out a form for each school, to be left with the principal.

3.0 HVAC Investigation

3.1 Investigate ventilation system and complete the HVAC survey with facility contact personnel.

3.2 Make certain that the fans are shut down so that close & safe observation of filters and coils can be made.

3.3 If there is evidence of mould growth, take a sample according to section 5.0 for analysis by the Provincial Laboratory of Public Health for Northern Alberta.

4.0 Visual inspection in selected rooms

4.1 Fill out the room survey. If mould growth is visible, obtain a tape sample according to section 5b for analysis by the Provincial Public Health Laboratory.

4.2 Take a picture of the room from the door.

5.0 Fungal Sampling

5a Water Samples (e.g., Humidifier reservoir)

5.1a If pooling of water is noticed, collect a sample in a specimen bottle for laboratory analysis.

5b Surface Samples – Clear adhesive-tape sampling[i]

When mould is suspected, follow the steps below:

5.1b Use latex gloves when handling tape.

5.2b Label the wax paper upon which the tape will be placed with a Sharpie pen, indicating identification number. Enter the date, time, school, phase, room, and identification number into a logbook.

5.3b Remove a 3 inch strip of tape from the dispenser. Handle tape by edges only.

5.4b Collect sample by gently touching the tape to a test surface and removing the tape with a slow, steady force. A sufficient sample will appear as a light deposit on the tape. Samples with too heavy a deposit will be difficult to examine by microscope and should be discarded into biological waste container. Make note of areas where the wall is porous and tape sample is difficult to obtain.

5.5b Take one blank on the first sampling day per week and submit this to the Provincial Public Health Laboratory.

5.6b Following sample collection, attach tape strips to wax paper (avoiding creases and folds).

5.7b Dipose of used latex gloves after each sample, placing them in a biological waste container.

5.8b Lay the wax paper flat in a Ziploc bag, then into the cooler for transportation to the Provincial Public Health Laboratory. Make certain that the ice pack does not come into contact with the agar strips.

6.0 Air sampling using RCS Plus[ii,iii,iv,v]

6.1 Don clean pair of disposable gloves.

6.2 Remove impeller drum and disinfect with isopropyl alcohol. Allow drum to dry.

6.3 Mark each agar strip blister pack with a unique sampling number using a Sharpie pen.

6.4 Handle agar strips by edges only. If agar is touched, strip should be discarded.

6.5 Peel back wrapper on agar strip by about 2cm and remove the agar strip. Inspect for any growth or contamination. Discard if any visible growth is observed.

6.6 Insert room temperature agar strip into the RCS Plus impeller assembly with agar surface facing towards the impeller blades until impeller drum is completely enclosed and the grip tab of the agar strip protrudes by about 2cm.
*6.7 Place the RCS Plus on a tripod, 3.3 ft (1 m) off the floor, near the center of the room, orient with impeller blades facing upwards towards the ceiling.
6.8 Turn instrument on to standby mode and check battery power. If battery power becomes insufficient during sampling the samples must be discarded.
6.9 Collect six-minute air samples with a sample volume of 300 L as recommended by Health Canada guidelines.
6.10 Using the grip tab, remove the agar strip from the RCS Plus impeller assembly, and place the strip back into its blister pack, nutrient surface facing downwards.
6.11 Seal sides of the blister pack with tape (being careful not to cover identification label), and place the sample into a Ziploc freezer bag.
6.12 Up to 10 agar strips in sealed blister packs can be placed into one Ziploc bag.
6.13 Ziploc bags should be returned to a dark cooler with an icepack as soon as possible after sampling. Rose Bengal agar is fungicidal with prolonged exposure to light.
6.14 After each sample, disinfect the RCS Plus impeller with isopropyl alcohol & air-dry.
6.15 Upon completion, place the RCS Plus back into its carrying case.
6.16 Deliver all samples to the Provincial Public Health Laboratory within 24 hours.
6.17 At the end of the day, remove the impeller drum and place in alcohol overnight. Allow to air dry.
6.18 Recharge batteries as necessary.

*If investigating within space variability, repeat steps 6.1 to 6.14 for the following locations:

- 1m away from the main door and 1m to the right (If a wall is directly to the right, place the Q-Trak 1m away to the left).
- 1m away from center of wall opposite from door

7.0 Velocicalc Plus

7.1 Using smoke tubes, determine if vent is supply or return. Note in logbook.
7.2 Place Velocicalc about 1" on the edge of the diffuser and perpendicular to the direction of air flow in order to obtain an accurate estimate of the flow rate of the incoming air from the duct. Make certain that the sensor window on the Velocicalc with the orientation dimple is facing upstream of the air current.
7.3 Collect replicate flow rate measurements from multiple locations in space served by each air supply diffuser or vent according to the table below:

Vent Type	Number of Measurements	Location of Measurement
Square diffuser	4	Middle plaque, center of side
Round diffuser	4	Middle cone, equally spaced
Rectangular grille (small -medium)	Varies	Middle of each 6" x 6" square
Rectangular grille (large)	Varies	Middle of each 12" x 12" square

8.0 Q-Trak Air Quality Monitor

8.1 Two Q-Traks will be used to sample randomly selected rooms. One will be used for an 8-hour measurement, the other will be used to obtain spot measurements of humidity, temperature, CO and CO_2. *Both Q-Traks will also be used to sample CO_2 decay in an unoccupied location over 2 hours.

ϒ8.2 For spot measurements, record data for 15 minutes. Place the Q-Trak 1m away from the main door and 1m to the right. If a wall is directly to the right, place the Q-Trak 1m away to the left. Ensure that the Q-Trak is placed 6.6 ft (2m) away from any occupant and in an area of the room where it will not be directly impacted by vents, dust, cold or heat sources. If this is not possible, note interferences in logbook.

8.3 Secure the Q-Trak instrument to sampling location with duct tape at an approximate height of 1m.

8.4 Record the locations of the Q-Trak in the logbook.

8.5 After the sample period, record average values for each parameter into the logbook. An interval of 15 minutes compares with ASHRAE standards at a height of 3.3ft (1m).

8.7 For the 8-hour sample, make certain that instrument is running off of A/C and not batteries. Furthermore, secure the handheld portion of the Q-Trak into the briefcase, and secure the briefcase to a non-moveable object (ie. Filing cabinet, desk).

8.8 Download information from Q-Trak onto a computer at the end of the day.

*For CO_2 decay subset, place the Q-Traks in 2 recently unoccupied classrooms according to instructions in section 8.2. Record the initial CO_2 reading in both classrooms. Record CO_2 data for 2 hours. Ensure school ventilation system is operating during sampling period.

ϒIf investigating within space variability, repeat steps 8.2 to 8.6 for the following locations:

- Near the center of the room
- 1m away from center of wall opposite from door

OUTDOOR INVESTIGATION SAMPLING PROTOCOL

Note weather, wind conditions, time of day and any outdoor activities that could affect results. Sampling during or after rain can skew outdoor data.

9.0 Outdoor Control Sampling

9.1 Obtain control sample for RCS Plus and the Q-Trak for temperatures > 0°C.
9.2 Take a control sample outdoors, 6-8 feet from the main entrance of the school, at a height of 3.3 ft (1 m).
9.3 Sample using RCS Plus and Q-Trak Plus according to section 11.0 and section 13.0 respectively.

10.0 Visual inspection

10.1 Inspect the outdoor air intake surroundings and respond to questions in the HVAC survey.
10.2 After ensuring that supply fan is back on, use smoke tubes to determine if air is flowing into outdoor air intake.
10.3 If water damage or pooling exists at the base of the OAI take a water sample according to section 5a.
10.4 If mould is visible, obtain a tape sample(s) according to the information provided from section 5b.

11.0 RCS Plus

11.1 This instrument cannot be operated at temperatures below 0°C, as the agar strips will freeze.
11.2 Repeat steps 6.1-6.17 from indoor RCS Plus air sampling
11.3 Using a tripod, place the RCS Plus vertically. Take sample 1ft away from the outdoor air intake that services the room(s) being sampled.
11.4 Shield outdoor fungal air samples from direct sunlight.

12.0 Velocicalc Plus

12.1 This instrument cannot be operated at temperatures below 5°C.
12.2 Use the grid method described in section 7.0 to sample outdoor air intakes (OAI). A ladder may be necessary. Follow safety procedures for ladders as listed in the *Portable Ladder Safety Checklist*.
12.4 Download data onto a computer at the end of the day.

13.0 Q-Trak

13.1 This instrument cannot be operated at temperatures below 5 °C
13.2 Take sample near OAIs 1.6 ft (0.5 m) away from the grating.
13.3 Take 15-minute spot measurements so that results may be compared to the ASHRAE 15-minute standard.
13.4 Download data onto a computer at the end of the day.

14.0 End of Day

14.1 Deposit tape and water samples for analysis at the Northern Provincial Public Health Laboratory

14.2 Obtain signature of receipt on Chain of Custody form.

14.3 Decontaminate sample containers with isopropyl alcohol in the staging area of EENV building.

14.4 Ensure all data has been successfully downloaded to computer.

14.5 Prepare investigation tools for next day.

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[i] Boss, M.J. and D.W. Day. (2001). *Air Sampling and Industrial Hygiene Engineering*. Lewis Publishers: Boca Raton, FL. 271 pp.

[ii] Boss, M.J. and D.W. Day. (2001). *Air Sampling and Industrial Hygiene Engineering*. Lewis Publishers: Boca Raton, FL. 271 pp.

[iii] American Conference of Governmental Industrial Hygienists (ACGIH). (1989). Guidelines for the Assessment of Bioaerosols in the Indoor Environment. (Burge, H.A., J.C. Freeley, K. Kreiss, D. Milton, P.R. Morey, J.A. Otten, K. Peterson, J.J. Tulius, R. Tyndall eds). ACGIH: Cincinnati, OH. 95 pp.

[iv] Sand, C. (2001). Technical Supervisor, National Centre for Mycology, University of Alberta Hospital, Edmonton, AB, May 2001, personal communication.

[v] American Industrial Hygiene Association Biosafety Committee (AIHA). (1996). *Field Guide for the Determination of Biological Contaminants in Environmental Samples*. (Dillon, H.K., P.A. Heinsohn, J.D. Miller eds). AIHA: Fairfax, VA. 174 pp.

Appendix 16. Indoor Air Quality Advisory

To Project Co-investigator: ☐ Lisa Johnstron (fax, 429-8436)
☐ Dale Lechelt (fax, 417, 8184)
☐ Arjen DeVries (fax, 467-5469)

From Project Investigators: Corinne, Mary and Lorelei

The following IAQ concerns were noted during our investigation and may warrant further attention by your department at this school.

School: _____

Type of IAQ Concern (√)		Room No.	Description of Concern
CO > 9 ppm (TWA 15 mins)			
CO_2 >2500 ppm (TWA 15 mins)			
Odours (e.g., gas, musty)			
HVAC system Deficiency			
Mould mass (in HVAC, or >1ft^2 on building mat'l)			
Water leakage			

Comments:

_____

_____

_____

Field Investigator: _____ **Date:** _____

Appendix 17. Raw Data

Key for Appendix 17

Group or Species	Key
Acremonium	1
Alternaria	2
Aspergillus flavus	3
A. fumigatus	4
A. glaucus "group"	5
A. niger	6
A. versicolor	7
Beauveria	8
Chaetomium	9
Cladosporium	10
Doratomyces	11
Epicoccum	12
Fusarium	13
Mucor	14
Non-sporulating	15
Paecilomyces	16
Penicillium	17
Phoma	18
Rhizopus	19
Scopulariopsis	20
Trichothecium	21
Ulocladium	22
Vertillicium	23
Yeast	24

Symbol	Definition
R	Room
C	Control
D	Duplicate
W	Within Space
-	Non-detectable

Code	ID	Fungal Species Present During Fall Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	4R	-	-	-	23	-	-	-	-	-	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5R	-	-	-	-	-	-	-	-	-	17	-	10	-	-	7	-	17	-	-	13	-	-	-	-
	6R	-	-	-	-	7	-	-	-	-	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7C	33	50	-	7	-	-	-	-	-	197	-	-	-	-	-	-	-	-	-	77	-	-	-	-
2	8R	-	33	-	-	-	-	-	-	-	33	-	-	-	-	27	-	-	-	-	-	-	-	-	-
	10R	-	123	-	-	-	-	-	-	-	13	-	-	-	-	7	-	7	-	-	-	-	-	-	-
	11R	-	27	-	-	-	-	-	-	-	20	-	13	-	-	-	-	-	-	-	-	-	-	-	-
	12C	-	-	-	-	13	-	-	10	-	20	-	-	-	-	13	-	3	-	-	-	-	-	-	-
3	13R	-	17	-	-	3	-	-	17	6	17	-	-	-	-	-	-	17	-	-	23	-	-	-	-
	14D	-	3	-	-	-	-	7	3	-	10	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	16aR	-	17	-	-	-	-	-	-	-	20	-	20	-	-	13	-	3	-	-	-	-	-	-	-
	16bR	-	20	-	7	-	-	7	-	-	-	-	-	-	-	-	-	30	-	-	-	-	-	3	-
	18C	-	20	-	7	43	-	-	-	-	33	-	-	-	-	13	-	13	-	13	40	-	-	-	-
4	19R	-	10	-	-	-	-	-	-	-	7	-	-	-	-	17	-	3	-	-	-	-	-	-	-
	20R	-	7	-	-	-	-	-	-	-	13	-	-	-	-	10	-	-	-	-	-	-	-	-	-
	21D	-	10	-	-	-	-	-	-	-	13	-	-	-	-	23	-	-	-	-	-	-	-	-	-
	22R	-	7	-	-	-	-	-	-	-	30	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	24C	-	13	-	-	37	-	-	-	-	27	-	-	-	-	-	-	13	-	-	23	-	-	-	-
12	25R	-	7	-	-	7	-	3	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-
	26D	-	7	-	-	-	-	-	-	-	-	-	-	-	-	7	-	3	-	-	-	-	-	-	-
	27R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	3	-	-	-	-	-	-	-
	28R	-	-	-	-	-	-	3	-	-	3	-	-	-	-	10	-	-	-	-	17	-	-	-	-
	29C	-	-	-	-	30	-	-	-	-	-	-	-	-	-	7	-	3	-	-	13	-	-	-	-
5	30R	-	30	-	7	-	-	-	-	-	33	-	-	-	-	20	17	-	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Fall Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
5	31D	-	7	-	-	-	17	10	-	-	17	-	-	-	-	13	10	-	-	-	17	-	-	-	-
	32R	-	-	-	-	-	-	-	10	-	13	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	33R	-	13	-	-	7	-	-	-	-	13	-	17	-	-	7	-	10	-	-	-	-	-	-	-
	34C	-	-	-	300	-	43	370	-	-	-	-	-	-	-	-	-	87	-	-	-	-	-	-	-
6	36R	-	13	-	-	-	-	-	-	-	17	-	-	-	-	17	-	-	-	67	-	-	-	-	-
	37D	-	-	-	-	-	13	13	-	-	40	-	-	-	-	17	-	10	-	-	-	-	-	-	-
	38R	-	20	-	-	-	-	-	-	-	27	-	-	-	-	20	-	-	-	-	-	-	-	-	-
	40R	-	-	-	-	-	-	3	-	-	13	-	-	-	-	10	-	-	-	-	-	-	-	-	-
	41R	-	7	-	-	-	-	-	-	-	27	-	-	-	-	17	-	-	-	-	-	-	-	-	-
	42C	-	-	-	-	13	-	13	-	-	30	-	-	-	-	-	-	67	-	-	-	-	-	-	-
	43R	-	23	-	-	-	-	-	-	-	37	-	-	-	-	20	-	10	-	-	-	-	17	-	-
7	44R	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	3	-	-	20	-	-	-	-
	45D	-	7	-	-	-	13	7	-	-	10	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	46R	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	47R	-	10	-	-	-	-	-	-	13	3	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	48R	-	-	-	-	-	-	-	-	-	10	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	49C	-	3	-	-	13	-	-	-	-	7	-	-	-	-	7	-	10	-	-	-	-	-	-	-
21	50R	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	223	-	-	-	-	-	-	-
	51R	-	-	-	-	-	-	-	-	-	17	-	-	-	-	10	-	27	-	-	-	-	-	-	-
	52D	-	-	-	-	-	-	-	-	-	13	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	53R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	54R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
8	55RW	-	23	-	-	-	3	-	-	-	-	-	-	-	-	3	-	13	-	-	-	-	-	-	-
	56RW	-	-	-	3	-	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Fall Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
8	57RW	-	13	-	-	-	-	-	-	-	-	-	-	-	-	13	-	3	-	-	-	-	-	-	-
	58RW	-	-	-	-	-	-	-	-	-	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	59RW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	60RW	-	-	-	3	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	61RW	-	16	-	-	-	-	-	-	-	-	-	4	-	-	16	-	-	-	-	-	-	-	-	-
	62RW	-	-	-	-	-	-	-	3	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	63RW	-	-	-	-	-	-	-	-	-	10	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	64C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
20	65R	-	-	-	-	3	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	66D	-	-	-	-	-	-	-	-	-	3	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	67R	-	10	-	3	-	-	-	-	3	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-
	68R	-	-	-	-	-	3	3	-	-	3	-	7	-	-	7	-	-	-	-	-	-	-	-	-
14	69R	-	-	-	-	-	-	10	-	-	13	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	70D	-	3	-	-	-	-	17	-	10	10	-	-	-	-	13	-	7	-	-	-	-	-	-	-
	72R	-	17	-	-	10	-	-	-	-	3	-	-	-	-	23	-	3	-	-	-	-	-	-	-
	73D	-	-	-	-	7	-	-	-	-	13	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	74R	-	-	-	-	-	13	7	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
18	75RW	-	-	-	-	-	-	-	-	-	53	-	-	7	-	-	-	3	-	7	-	-	-	-	-
	76DW	-	-	-	-	-	-	-	-	-	20	-	-	-	-	13	-	-	-	-	-	-	-	-	-
	77RW	-	20	-	-	-	-	-	-	-	30	-	-	-	-	10	3	-	-	-	-	-	-	-	-
	78RW	-	10	-	-	-	-	-	-	-	20	-	-	-	-	13	-	-	-	-	7	-	-	-	-
	79RW	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	13	-	-	-	-
	80RW	-	17	-	-	-	-	-	-	-	10	-	-	-	-	6	-	-	-	-	-	-	-	-	-
	81RW	-	-	-	-	-	-	7	-	-	13	-	-	-	-	-	-	3	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Fall Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
18	82RW	-	-	-	-	-	-	-	-	-	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	83RW	-	-	-	-	-	-	-	-	-	27	-	-	-	-	13	-	3	-	-	-	-	-	-	-
	84RW	-	-	-	-	-	-	-	-	-	17	-	-	-	-	-	-	6	-	-	-	-	-	-	-
	85C	-	-	-	-	-	-	-	-	-	617	-	-	-	-	73	-	13	-	-	-	-	-	-	-
13	86RW	-	3	-	-	13	-	3	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	87DW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-
	88RW	-	-	-	-	-	-	-	-	-	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	89RW	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	90RW	-	-	-	40	7	-	-	-	-	10	-	-	-	-	30	-	-	-	-	-	-	-	-	-
	91RW	-	-	-	17	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	92RW	-	-	-	3	-	-	-	-	-	3	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	93RW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	94RW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	95aRW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	95bC	-	-	-	17	57	-	-	-	-	60	-	-	-	-	-	-	47	-	-	13	-	-	-	-
15	96RW	-	-	-	13	-	-	30	-	-	-	-	-	-	-	-	-	7	-	-	20	-	-	-	-
	97DW	-	20	-	-	-	-	-	-	-	10	-	-	-	-	3	-	7	-	-	7	-	-	-	-
	98RW	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	3	-	3	-	-	-	-	-
	99RW	-	7	-	-	-	-	-	-	-	17	-	-	-	-	3	-	-	-	-	13	-	-	-	-
	100RW	-	-	-	-	10	-	-	-	-	3	-	-	-	-	-	-	7	-	-	-	-	-	-	-
	101RW	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	7	-	7	-	-	-	-	-
	102RW	-	-	-	13	3	-	-	-	-	3	-	-	-	-	-	-	3	-	-	-	-	-	-	-
	103RW	-	13	-	-	17	-	-	-	-	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	104RW	-	7	-	20	17	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Fall Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
15	105RW	-	-	-	17	6	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-	-
	106C	-	-	-	33	77	-	7	-	-	-	-	-	-	-	-	-	17	-	-	33	-	-	-	-
17	107R	-	3	-	-	3	-	-	-	-	20	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	108D	-	-	-	-	-	-	-	-	-	10	-	-	-	-	10	-	-	-	-	-	-	-	-	-
	109R	-	6	-	-	-	-	-	-	-	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	110R	-	-	-	-	-	-	-	-	-	7	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	112C	-	10	-	13	33	-	-	-	-	367	-	-	-	-	30	-	53	-	-	7	-	-	-	-
16	114RW	-	7	-	-	-	-	10	-	-	10	-	-	-	-	13	-	-	-	-	-	-	-	-	-
	115RW	-	-	-	-	-	-	-	-	-	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	116aRW	-	-	-	-	3	-	7	3	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	116bRW	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	3	-	-	-	-	-	-
	117RW	-	-	-	-	3	-	-	-	-	-	-	-	-	-	10	-	3	-	-	-	-	-	-	-
	118RW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	3	-	-	-	-	-
	119RW	-	-	-	-	-	-	-	-	-	17	-	7	-	-	13	-	3	-	-	-	-	3	-	-
	120RW	-	13	-	-	3	-	-	-	-	3	-	-	-	-	10	-	-	-	-	-	-	-	-	-
	121RW	-	-	-	-	-	-	-	-	-	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	122C	-	-	-	-	20	-	27	-	-	20	-	-	-	-	20	-	10	-	-	33	-	43	-	-
19	123RW	-	13	-	-	7	-	-	-	-	7	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	124DW	-	-	-	13	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	125RW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	126RW	-	13	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	127RW	-	-	-	-	3	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	128RW	-	-	-	-	-	-	-	-	-	16	-	-	-	-	16	-	7	-	-	-	-	-	-	-
	129RW	-	-	-	-	-	-	-	-	-	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Fall Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
19	130RW	-	-	-	-	-	13	-	7	-	7	3	-	-	-	10	-	7	-	-	-	-	-	-	-
	131RW	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	132RW	-	7	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	133C	-	10	-	23	40	-	13	-	-	107	-	-	-	-	40	-	13	-	-	-	-	-	-	-
11	134R	-	-	-	-	3	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	135D	-	-	-	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	136R	-	13	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	137R	-	13	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-	-
9	138RW	-	-	-	-	-	-	7	-	-	183	-	-	-	-	20	-	-	-	-	-	-	-	-	-
	139DW	-	3	-	-	-	-	3	-	-	57	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	140RW	-	3	-	-	-	-	-	-	-	37	-	-	-	-	17	-	-	-	-	-	-	-	-	-
	141RW	-	-	-	-	-	-	-	10	13	50	-	-	-	-	10	-	-	-	-	-	-	17	-	-
	142RW	-	13	-	-	-	-	-	-	-	17	-	3	-	-	-	-	-	-	-	-	-	-	-	-
	143RW	-	-	-	-	-	-	-	-	-	-	-	17	-	-	13	-	-	-	-	-	-	-	-	-
	144RW	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	145RW	-	-	-	-	-	-	-	-	-	47	-	-	-	-	17	-	-	-	-	-	-	-	-	-
	146RW	-	-	-	-	-	-	-	3	-	63	-	-	-	-	17	-	-	-	-	-	-	-	-	-
	147RW	-	20	-	-	-	-	-	-	-	67	-	-	-	-	30	-	-	-	-	-	-	-	-	-
	149C	-	13	-	-	77	-	-	-	-	67	-	-	-	-	-	-	-	-	7	10	13	-	-	-
22	150RW	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	20	-	-	-	-	-
	151DW	-	10	-	-	-	-	-	-	-	23	-	-	-	-	3	-	20	-	7	-	-	-	-	-
	152RW	-	-	-	-	-	-	17	-	-	-	-	-	-	-	-	-	40	-	160	-	-	-	-	-
	154RW	-	-	-	-	-	-	10	-	-	-	-	-	-	-	17	-	20	-	43	-	-	-	-	-
	155RW	-	-	-	-	-	13	-	-	-	33	-	10	-	-	-	-	40	-	43	-	-	-	-	-

Code	ID	Fungal Species Present During Fall Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
22	156RW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17	-	37	-	87	-	-	-	-	-
	153RW	-	10	-	-	-	3	3	-	-	43	-	-	-	-	13	-	60	-	57	-	-	-	-	-
	157aC	-	10	-	30	-	20	20	-	-	33	-	-	-	-	23	-	13	-	37	-	-	-	-	-
	157bRW	-	7	-	-	-	-	-	-	-	47	-	-	10	-	-	-	7	-	7	-	-	-	-	-
	158RW	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17	-	3	-	-	-	-	-
	159RW	-	10	-	7	-	3	-	-	-	40	-	-	-	-	17	-	-	-	33	-	-	-	-	-
10	160RW	-	-	-	-	-	7	-	-	-	27	-	-	-	-	-	-	273	-	-	10	-	-	-	-
	161RW	-	-	-	-	-	17	-	-	-	-	-	-	-	-	-	-	580	-	-	20	-	33	-	-
	162RW	-	13	-	-	-	-	-	-	-	13	-	-	-	-	-	-	70	-	-	-	-	-	-	-
	163RW	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	183	-	-	-	-	-	-	-
	164RW	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	200	-	-	17	-	-	-	-
	165RW	-	-	-	-	-	7	20	-	-	-	-	-	-	-	33	-	120	-	-	-	-	-	-	-
	166RW	-	-	-	-	-	10	20	-	-	10	-	-	-	-	67	-	87	-	-	-	-	-	-	-
	167RW	-	20	-	-	-	-	-	-	-	50	-	-	-	-	50	-	67	-	-	-	30	-	-	-
	168C	-	-	-	23	83	-	47	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-

Code	ID	Fungal Species Present During Winter Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
23	2R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5R	-	-	-	-	-	-	7	7	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-
	6R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7C	-	-	-	30	250	-	-	-	-	-	-	-	-	-	30	-	120	-	-	100	-	-	-	-
24	8R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	11R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	12R	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	13R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	14R	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	15C	-	-	-	-	90	-	-	-	-	-	-	-	-	-	10	-	10	-	-	20	-	-	-	-
25	16C	-	-	-	10	60	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-
	17R	7	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	19D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20R	-	-	-	7	-	-	-	-	-	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-
7	21R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22D	-	-	-	37	-	-	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	23R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	24R	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-
	26R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Winter Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
7	27D	-	-	-	10	50	-	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	28R	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	29D	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	30R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	30	-	-	-	-	-
	31R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	32R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
	33R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	34C	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-
4	35R	-	-	-	-	-	-	-	-	-	-	-	-	-	47	-	-	-	-	-	-	-	-	-	-
	36D	-	-	-	3	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	38R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	39R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	40R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	41R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	42C	-	-	-	10	100	-	60	20	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	43R	-	-	-	17	3	-	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	44D	-	-	-	-	-	-	23	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	45R	-	-	-	-	-	23	-	-	-	-	-	-	-	-	20	-	27	-	27	-	-	-	-	-
	46R	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	47R	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	48R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	49C	-	-	-	-	100	-	40	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	50R	-	-	-	-	-	-	3	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	51D	-	-	-	10	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Winter Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
28	52R	-	-	-	-	-	-	10	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	53R	-	-	-	3	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	54R	-	-	-	3	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	55R	-	7	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	56C	-	-	-	30	-	-	-	-	-	20	-	-	-	-	-	-	10	-	-	40	-	-	-	-
29	57R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	58D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	59R	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	60R	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	61R	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	62C	-	-	-	20	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	-	-	-	-
2	63R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	64D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	65R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	66R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	67C	-	-	-	-	37	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	68R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	69R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	70D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	71R	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	72R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	150	-	-	-	-	-	-	-
	73R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	74C	-	-	-	-	67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	75R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Winter Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
31	76R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	77D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	78R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-
	79R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	160	-	-	-	-	-	-	-
	80R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-
	81C	-	-	-	13	10	-	-	-	-	13	-	-	-	-	-	-	-	-	-	10	-	-	-	-
1	82R	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	83R	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-
	84R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	85C	-	-	-	-	10	-	-	-	-	-	-	-	-	-	10	-	20	-	-	-	-	-	-	-
6	86R	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-
	87R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	88R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	89R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	90C	-	-	-	10	47	-	20	-	-	-	-	-	-	-	17	-	3	-	-	7	-	-	-	-
32	90R	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	91D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	92R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	93R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	94R	-	-	-	3	-	-	80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	95R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	96C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	97R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	98R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Winter Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
21	99R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	100D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	101C	-	-	-	-	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	102R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	103D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	104R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	105R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	106R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	107R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	108C	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-
3	109R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	110D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
	111R	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	112R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	113C	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	114R	-	-	-	-	-	-	3	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	115D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	116R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	117R	-	10	-	-	-	27	10	-	-	-	-	-	-	-	20	-	50	-	-	-	-	-	-	-
	119R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60	-	-	-	-	-
	120C	-	-	-	-	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	121R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	122R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	123R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Winter Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
20	124D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	125C	-	-	-	-	50	-	30	-	-	-	-	-	-	-	20	-	-	-	-	-	-	-	-	-
35	126R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	127D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	128R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	129R	-	-	7	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	130R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	131R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	132C	-	-	20	-	20	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-
14	133R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
	134D	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
	135R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	136R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	137C	-	-	-	-	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	137R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	138D	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	139R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	140R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	141R	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	142C	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	143R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	144R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	145D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	146R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Winter Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
11	147C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	148R	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	149D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	150R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	151R	-	-	-	-	-	3	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-
	152R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17	-	-	-	-	-	-	-
	153R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	154C	-	-	-	-	30	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-
38	155R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	156D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	157R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	158R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	159R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	160R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	161C	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	162R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	163D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	164R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	165R	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	166R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	-	33	-	-	-	-	-
	167R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	168C	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	169R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	170D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Code	ID	Fungal Species Present During Winter Sampling (CFU/m^3)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
40	171R	-	-	-	-	37	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	172R	-	3	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	173R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	174R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	175C	-	-	-	-	77	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	176R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	177R	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	178D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	179R	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	180R	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	181R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	17	-	-	-	3
	182C	-	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	183R	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	184D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	185R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	186R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	187R	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	188R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	189C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	190R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	191D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	192R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	193R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	193aR	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-

Code	ID	Fungal Species Present During Winter Sampling (CFU/m^3)																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
43	194D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-
	195C	-	-	-	-	130	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 18. Statistical Test for Duplicate Differences

Wilcoxon's Matched Paired Non-Parametric Test
Hypothesized difference of fungal counts between duplicate samples
Significance Level $\alpha = 0.05$
Program Used = SPSS 8.0 for Windows Student Version

Species	Rank	Observations	Mean Rank	Sum of Ranks	Test Statistic
Cladosporium	positive rank	9	8.11	73.0	$\alpha = 0.459$
	negative rank	6	7.83	47.0	
sample 1 -	ties	3			
sample 2	total	18			
Non-sporulating	positive rank	7	9.21	64.5	$\alpha = 0.855$
	negative rank	9	7.94	71.5	
sample 1 -	ties	2			
sample 2	total	18			

Appendix 19. Statistical Test for Indoor-Outdoor Differences

Wilcoxon's Matched Paired Non-Parametric Test
Hypothesized difference of fungal counts between indoor and outdoor locations
Significance Level $\alpha = 0.05$
Program Used = SPSS 8.0 for Windows Student Version

Species	Rank	Observations	Mean Rank	Sum of Ranks	Test Statistic
Cladosporium	positive rank	5	7.0	35.0	$\alpha = 0.049$
	negative rank	12	9.83	118	
indoor - outdoor	ties	1			
	total	18			
Non-sporulating	positive rank	10	7.45	74.5	$\alpha = 0.409$
	negative rank	5	9.10	45.5	
indoor - outdoor	ties	3			
	total	18			

Appendix 20. Statistical Test for Fall-Winter Differences

Wilcoxon's Matched Paired Non-Parametric Test
Hypothesized difference of fungal counts between fall and winter
Significance Level $\alpha = 0.05$
Program Used = SPSS 8.0 for Windows Student Version

Species	Rank	Observations	Mean Rank	Sum of Ranks	Test Statistic
Cladosporium	positive rank	22	11.5	35.0	$\alpha = 0.00$
	negative rank	0	0	0	
fall - winter	ties	8			
	total	30			
Non-sporulating	positive rank	23	13.0	298	$\alpha = 0.00$
	negative rank	1	2.00	2.00	
fall - winter	ties	6			
	total	30			

Appendix 21. Within Space Variability

Appendix 21.1: Within Space Variability for School #10, Room A
Fungal Count (CFU/m³)
600
500
400
300
200
100
0
Ulocladium
Scopulariopsis
Penicillium
Cladosporium
Aspergillus niger
Alternaria
1
2
3
Location

Appendix 21.2: Within Space Variability for School #10, Room C

Appendix 21.3: Within Space Variability for School #2, Room A
Fungal Count (CFU/m³)
160
140
120
100
80
60
40
20
0
Rhizopus
Penicillium
Non-sporulating
Cladosporium
Aspergillus versicolor
Alternaria
1
2
3
Location

Appendix 21.4: Within Space Variability for School #22, Room B

Appendix 21.5: Within Space Variability for School #22, Room C

Appendix 21.6: Within Space Variability for School #9, Room A

Appendix 21.7: Within Space Variability for School #9, Room B

Appendix 21.8: Within Space Variability for School #9, Room C
Fungal Count (CFU/m³)
70
60
50
40
30
20
10
0
Non-sporulating
Cladosporium
Beauveria
Alternaria
1
2
3
Location

Appendix 21.9: Within Space Variability for School #19, Room A
Fungal Count (CFU/m³)
14
12
10
8
6
4
2
0
Non-sporulating
Cladosporium
Aspergillus versicolor
Aspergillus glaucus "group"
Aspergillus fumigatus
Alternaria
1
2
3
Location

Appendix 21.10: Within Space Variability for School #19, Room B

Appendix 21.11: Within Space Variability for School #19, Room C

Appendix 21.12: Within Space Variability for School #16, Room A

Appendix 21.13: Within Space Variability for School #16, Room B

Appendix 21.14: Within Space Variability for School #16, Room C
Fungal Count (CFU/m³)
30
25
20
15
10
5
0
Ulocladium
Penicillium
Non-sporulating
Epicoccum
Cladosporium
Aspergillus glaucus "group"
Alternaria
1
2
3
Location

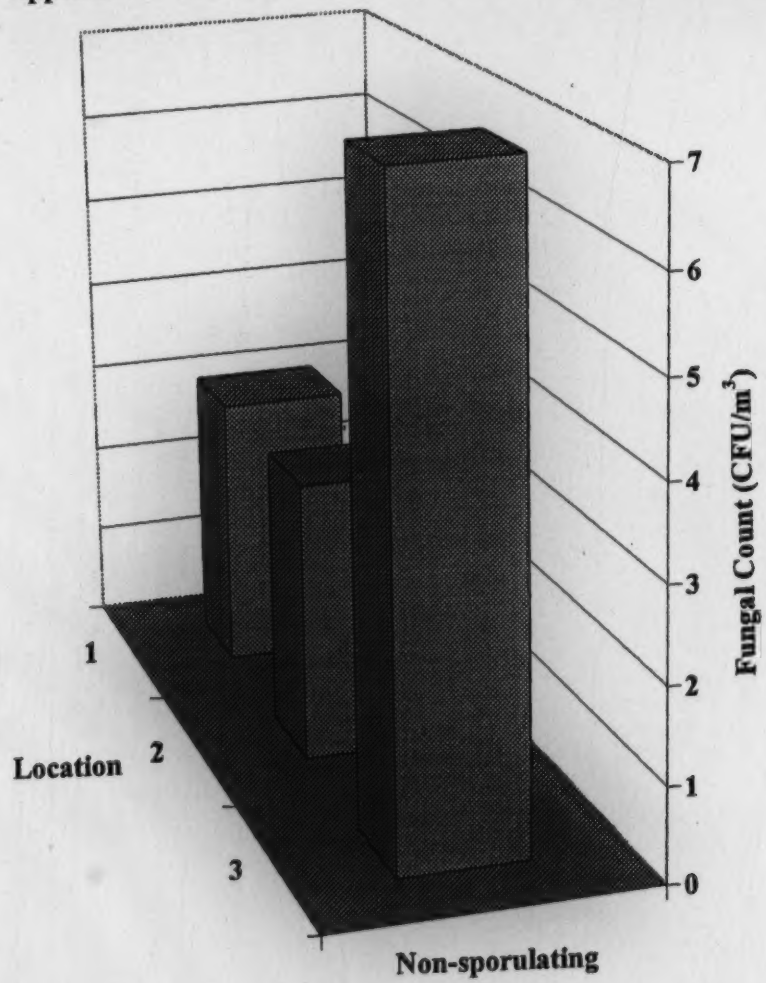
Appendix 21.15: Within Space Variability for School #15, Room A

Appendix 21.16: Within Space Variability for School #15, Room B
Fungal Count (CFU/m³)
14
12
10
8
6
4
2
0
Rhizopus species
Penicillium species
Cladosporium species
Aspergillus glaucus "group"
Aspergillus fumigatus
Location
1
2
3

Appendix 21.17: Within Space Variability for School #13, Room A

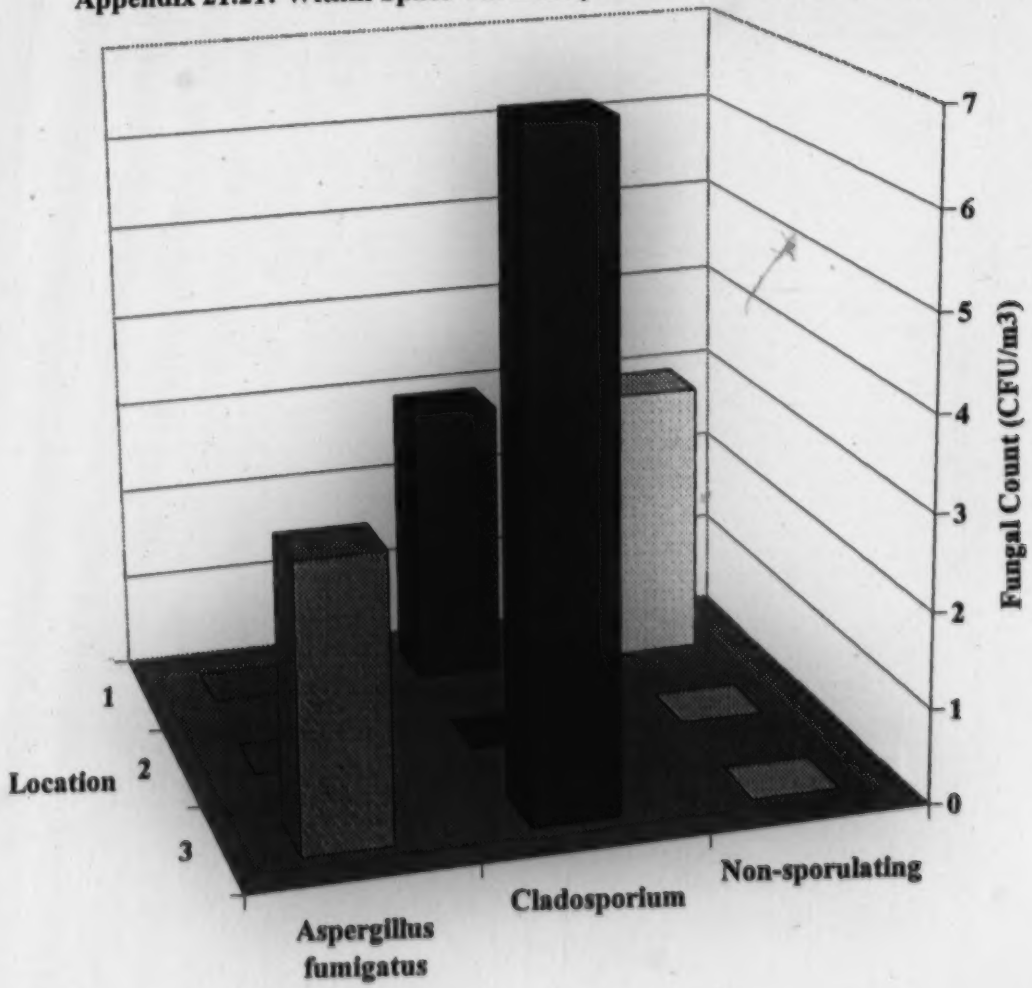
Appendix 21.18: Within Space Variability for School #13, Room B

Appendix 21.19: Within Space Variability for School #13, Room C



Appendix 21.20: Within Space Variability for Within School #8, Room A
Fungal Count (CFU/m³)
25
20
15
10
5
0
Location
1
2
3
Alternaria
Aspergillus fumigatus
Aspergillus niger
Non-sporulating
Penicillium

Appendix 21.21: Within Space Variability for Within School # 8, Room B

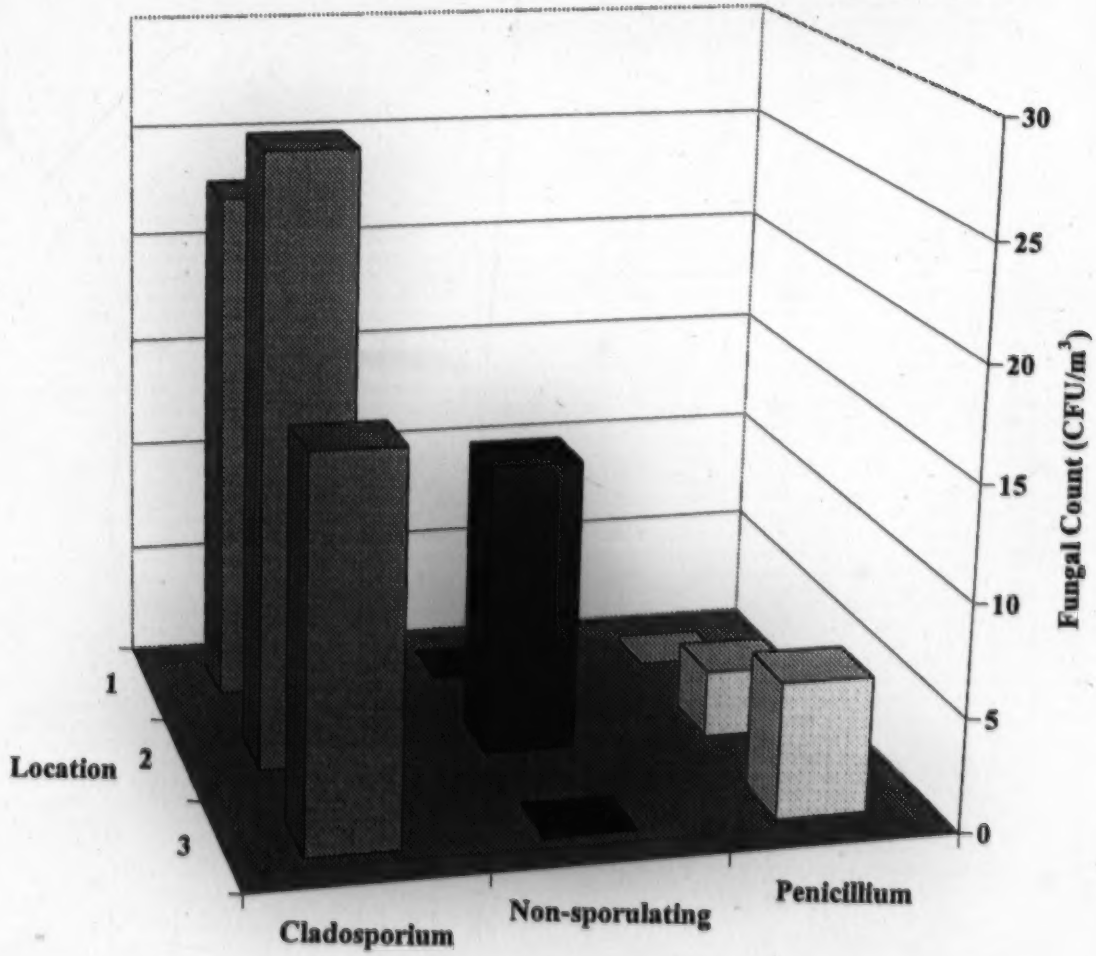


Appendix 21.22: Within Space Variability for School #8, Room C
Fungal Count (CFU/m³)
16
14
12
10
8
6
4
2
0
Non-sporulating
Epicoccum
Cladosporium
Beauveria
Alternaria
1
2
3
Location

Appendix 21.23: Within Space Variability for School #18, Room A

Appendix 21.24: Within Space Variability for School #18, Room B

Appendix 21.25: Within Space Variability for School #18, Room C



Appendix 22. Statistical Test for Within Phase Differences

Wilcoxon's Matched Paired Non-Parametric Test
Hypothesized difference of fungal counts within rooms of same phase
Significance Level $\alpha = 0.05$
Program Used = SPSS 8.0 for Windows Student Version

Species	Rank	Observations	Mean Rank	Sum of Ranks	Test Statistic
Cladosporium	positive rank	9	7.72	69.5	$\alpha = 0.589$
	negative rank	6	8.42	50.5	
room A -Room B	ties	4			
	total	19			
Non-sporulating	positive rank	9	8.61	77.5	$\alpha = 0.622$
	negative rank	7	8.36	58.5	
room A -Room B	ties	3			
	total	19			

Appendix 23. Statistical Test for Between Phase Differences

Wilcoxon's Matched Paired Non-Parametric Test
Hypothesized difference of fungal counts between rooms of different phases
Significance Level $\alpha = 0.05$
Program Used = SPSS 8.0 for Windows Student Version

Species	Rank	Observations	Mean Rank	Sum of Ranks	Test Statistic
Cladosporium	positive rank	7	4.0	28.0	$\alpha = 0.018$
	negative rank	0	0	0	
room A Phase I -	ties	4			
room B Phase II	total	11			
Non-sporulating	positive rank	6	6.25	37.5	$\alpha = 0.715$
	negative rank	4	4.38	17.5	
room A Phase I -	ties	1			
room B Phase II	total	11			